

On the Peripheral Nervous Control of the Lower Urinary Tract

by

Anders Mattiasson



Lund 1984



From the Departments of Clinical Pharmacology and Urology, University of
Lund, Sweden

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To Elisabet

The present thesis is based on the following papers, which in the text are referred to by their roman numerals (I–VIII)

- I Mattiasson A, Andersson K-E and Sjögren C:
Adrenergic and non-adrenergic contraction of isolated urethral muscle from rabbit and man.
Submitted to Journal of Urology.
- II Andersson K-E, Mattiasson A and Sjögren C:
Electrically induced relaxation of the noradrenaline contracted isolated urethra from rabbit and man.
Journal of Urology 1983, 129:210–214.
- III Mattiasson A, Andersson K-E and Sjögren C:
Adrenoceptors and cholinceptors controlling the release of noradrenaline from adrenergic nerves in the isolated urethra from rabbit and man.
Journal of Urology (In press).
- IV Sjögren C, Andersson K-E and Mattiasson A:
Effects of vasoactive intestinal polypeptide on isolated urethral and urinary bladder smooth muscle from rabbit and man.
Submitted to Journal of Urology.
- V Mattiasson A, Andersson K-E and Sjögren C:
Contractant and relaxant properties of the female rabbit urethral submucosa.
Submitted to Journal of Urology.
- VI Mattiasson A, Andersson K-E and Sjögren C:
Urethral sensitivity to α -adrenoceptor stimulation and blockade in patients with parasympathetically decentralized lower urinary tract and in healthy volunteers.
Neurourology and Urodynamics (In press).
- VII Andersson K-E, Ek A, Hedlund H and Mattiasson A:
Effects of prazosin on isolated human urethra and in patients with lower motor neuron lesions.
Investigative Urology 1981, 19:39–42.
- VIII Mattiasson A, Andersson K-E, Sjögren C, Sundin T and Uvelius B:
Supersensitivity to carbachol in the parasympathetically decentralized feline urinary bladder.
Journal of Urology (In press).



CONTENTS

Introduction	9
<i>The urethra</i>	
Functional anatomy of the urethra	
Innervation	
Adrenoceptor mediated functions	
Cholinoceptor mediated functions	
Non-adrenergic, non-cholinergic functions	
<i>Parasympathetic decentralization</i>	
The aims of the present study	13
Material and methods	14
<i>In vitro investigations</i>	
Dissection and preparation	
Treatment of rabbits with 6-hydroxydopamine	
Functional investigations	
Electrical field stimulation	
Release studies	
Measurement of noradrenaline	
Light and electron microscopy	
<i>In vivo investigations</i>	
Selection of patients	
Urethral pressure profiles	
Surgical procedures	
<i>Statistical analysis</i>	
Results	17
I–VIII	
General Discussion	27
<i>The urethra</i>	
Adrenoceptor mediated functions	
Cholinoceptor mediated functions	
Adrenergic-cholinergic interaction	
Non-adrenergic, non-cholinergic functions	
Contraction	
Relaxation	
Neuropeptides	
Vasoactive intestinal polypeptide (VIP)	
Neuropeptide Y (NPY)	
<i>Parasympathetic decentralization</i>	
Summary	32
Acknowledgements	33
References	34
Papers I–VIII	43

Introduction

The urethra

A normal urethral function is expected to guarantee urinary continence both at rest and during stress conditions, and also to allow the urine to pass with the least possible resistance during micturition.

Urinary continence at rest is to a great part maintained by a high and stable intraurethral pressure. The maximum urethral pressure at rest is an expression for both active and passive forces, i.e. muscle contraction, hydrostatic factors and tissue elasticity (Rud et al., 1980). The urethral pressure increase secondary to an increase of the intraabdominal pressure depends in part on transmission of the intraabdominal pressure to the proximal part of the urethra (Enhörning, 1961). The pressure increase in the urethra seems to begin before the pressure increase in the bladder and is often higher in the urethra than in the bladder (Constantinou & Govan, 1980; Thüroff et al., 1982). Therefore the presence also of an active, reflexogenic closure mechanism in response to stress cannot be excluded (Constantinou & Govan, 1980; Faysal et al., 1981; Thüroff et al., 1982).

During micturition a pronounced decrease of the urethral pressure is seen, beginning a few seconds before the detrusor starts contracting (Tanagho & Miller, 1970). It is probable that the pressure decrease not only is the result of relaxation of striated muscle components but also of urethral smooth muscle (see, e.g. Zinner et al., 1976). On the other hand, it cannot be excluded that contractant components contribute to the opening of the urethra during micturition (Tanagho et al., 1969; Zinner et al., 1976).

Functional anatomy of the urethra

The most important continence-maintaining part of the urethra at rest is the zone where the maximum urethral pressure is represented. In the human female urethra this zone is found in the middle third of the organ, while in the male urethra, where it might be called the distal sphincteric mechanism (Turner-Warwick, 1975), it is located in the infracollicular part of the prostatic urethra and in the membranous urethra. The morphological structures of the urethral wall comprising these zones in man, are striated and smooth muscle layers, surrounding the mucosal and submucosal (lamina propria) layers.

The striated and smooth muscle components comprise separate layers of the human urethral wall. The striated layer is often called "the external sphincter" (Gosling et al., 1982). In the female it extends along the whole urethra with the thickest portion in the middle third of the organ. The striated muscle layer is distributed in a non-symmetrical way with an anteriorly and laterally located predominance (Gosling et al., 1982). In the male urethra the striated muscle is confined to the area of the distal sphincteric mechanism (Turner-Warwick & Chilton, 1981). The striated muscle of the urethral wall does not morphologically constitute an integral part of the pelvic floor (Turner-Warwick & Chilton, 1981). The influence of the striated muscle components on the urethral pressure varies in different investigations from almost none to more than 50% of the maximum urethral pressure (Lapides, 1957; Tanagho et al., 1969; Donker et al., 1972; Awad & Downie, 1976; Rud et al., 1980). Lapides showed 1957, that micturition could be initiated and urinary flow stopped voluntarily under striated muscle paralysis with curare.

Several investigators have stressed the importance of the urethral smooth muscle for the maintenance of urinary continence (Tanagho et al., 1969;

Donker et al., 1972; Awad & Downie, 1976). In man the urethral smooth muscle is distributed along the whole length of the urethra with an inner longitudinal and an outer circular layer (c.f. van Geelen, 1983), which seem to be separated from the smooth muscle of the bladder (van den Bulcke et al., 1970; Dröes, 1974; Gosling et al., 1982). The functional significance of the arrangement of the urethral smooth muscle in different layers has not been established.

It cannot be excluded that other components of the urethral wall than the described muscle layers also actively contribute to the urethral function (Berkow & Amboy, 1953). The richly vascularized submucosal layer is a quantitatively important structure of the urethral wall. The mucosal and submucosal lining of the urethra has been ascribed passive properties and the expression "inner urethral softness" was introduced to describe the functional role of these structures in the maintenance of urinary continence (Zinner, 1976, 1980, 1983a, b). The possibility that the submucosal tissue possesses active contractile properties of possible importance for the urethral sphincteric function has not been investigated.

Innervation

The lower urinary tract is innervated by sympathetic, parasympathetic and somatic nerves. The sympathetic system mediates contraction of the bladder outlet and urethra (Learmonth, 1931), while the parasympathetic innervation mediates cholinergic and in several species also non-adrenergic non-cholinergic contraction of the bladder (Ambache & Zar, 1970; Downie & Dean, 1977). A reciprocal inhibitory function of the two systems has been suggested (e.g. Elliot 1906–1907).

The adrenergic, hypogastric, nerves originate in the thoracolumbar sympathetic outflow of the spinal segments Th₁₀–L₂ (Bors & Comar, 1971; Fletcher & Bradley, 1978). The preganglionic neurons form synapses with the adrenergic neurons in the inframesenteric and hypogastric ganglia (Fletcher & Bradley, 1978). There are probably also sympathetic ganglionic cells adjacent to or in the walls of the target organs, since the presence of short adrenergic neurons in the smooth muscle of the bladder and the urethra have been described (see e.g. Elbadawi, 1982). A population of adrenergic preganglionic neurons innervating short adrenergic neurons have recently also been found (Elbadawi, 1984). The parasympathetic innervation of the smooth muscle of the bladder and urethra comes from the spinal segments S_{3–4} and is mediated in the pelvic nerves (Bradley et al., 1974; Guntenberg et al., 1975). The parasympathetic relay stations are located in pelvic ganglia outside the bladder and the urethra but also in the walls of these organs (Elbadawi, 1982).

The somato-motor innervation of the striated muscle of the pelvic floor is conveyed in the pudendal nerves, which originate in the spinal segments S_{2–4}. The innervation of the striated muscle of the urethral wall ("external sphincter") seems to be less clear. Some investigators have described this muscle to be supplied by somatic nerve fibers in the pelvic nerves (Gilvernet, 1964; Donker et al., 1976; Gosling et al., 1977) while others mean that the somato-motor innervation is mediated via the pudendal nerves (e.g. Tanagho et al., 1982).

The neurons found at microscopic investigation of both the bladder and the urethra are thus adrenergic and cholinergic, but also non-adrenergic, non-cholinergic. The latter comprise a population of less well defined neurons containing larger neuro-vesicles than the adrenergic and cholinergic nerve

fibers. The nature of the transmitter(s) in these neurons is not known but both purines and peptides have been suggested (see e.g. Andersson & Sjögren, 1982).

Adrenoceptor mediated functions

The adrenergic innervation of the urethral smooth muscle is especially rich within the preprostatic part of the organ (Ek et al., 1977). In the rest of the male and in the female urethra the occurrence of adrenergic nerve fibers is less rich, but with a uniform distribution (Ek et al., 1977).

The adrenoceptors in the urethra are of both α - and β -type. In man, α -adrenoceptor agonists increase the intraurethral pressure while α -adrenoceptor blocking agents reduce the pressure (Donker et al., 1972; Awad & Downie, 1976; Ek et al., 1978). In vitro, corresponding results are found; human and rabbit urethral smooth muscle preparations are contracted by α -adrenoceptor agonists, and these responses can be blocked by means of α -adrenoceptor blocking drugs (Ek et al., 1977; Andersson et al., 1984). β -adrenoceptor mediated relaxation can be demonstrated in the human urethra both in vivo and in vitro (c.f. Andersson & Sjögren, 1982). Quantitatively, the dominating activity in the urethra induced by noradrenaline (NA) is mediated via α -adrenoceptors and α -adrenoceptor stimulating and blocking agents are widely used in clinical practice to increase and decrease the intraurethral pressure, respectively (see e.g. Norlén, 1982). Therefore, it seemed to be of great interest to further investigate the α -adrenoceptor mediated activity with regard both to the possible presence of different subtypes of these receptors (α_1 and α_2) within the urethra and also their pre- and postjunctional distribution.

The α -adrenoceptor subtype distribution in the human urethra has not been investigated. In the female rabbit urethra the presence of postjunctionally located contraction-mediating α_1 - and α_2 -adrenoceptors has recently been demonstrated (Andersson et al., 1984). The total amount of α -adrenoceptors was also determined, using radioligand binding technique. The relation between α_1 - and α_2 -adrenoceptors was found to be approximately 1:3.

In several organs with adrenergic innervation prejunctionally located α -adrenoceptors mediating a negative feed-back (autoregulation) of the release of NA have been demonstrated (Starke, 1977; Langer, 1981). Prejunctionally located α -adrenoceptors have been demonstrated also on other than adrenergic nerve terminals, e.g., in the intestine where they inhibit the release of acetylcholine (ACh) from cholinergic neurons (Wikberg, 1981). An interaction between adrenergic and cholinergic nerves not only at a ganglionic level (DeGroat & Saum, 1972) but also at the nerve terminal level has been suggested to occur in the lower urinary tract on the basis of morphological findings (Elbadawi, 1982) and in vivo investigations on animals (McGuire & Herlihy, 1979). Whether a receptor-basis for such an interaction is present also in the urethra is not known.

Cholinoceptor mediated functions

The functional role of the relatively rich cholinergic innervation in the urethra is not completely understood. It is known from in vitro-investigations in different animal species that cholinoceptor stimulating agents mediate contraction of longitudinally oriented urethral preparations (Persson & Andersson, 1976; Hassouna et al., 1983), and, in an investigation on the proximal male rat urethra, also of circularly oriented preparations (Ekström & Malmberg, 1983). The discrepancy between the relatively rich occurrence of cholinergic nerves in

the urethra and the functional in vitro and in vivo findings suggests an influence of the cholinergic innervation also on other structures than the urethral muscle, such as, e.g., other types of neurons. This is supported by the morphological findings of a close connection between cholinergic and adrenergic nerves in the urethra (Elbadawi, 1982).

Non-adrenergic, non-cholinergic functions

Neuropeptides

Several neuropeptides have been suggested as putative non-adrenergic, non-cholinergic transmitters or neuromodulators in the lower urinary tract (Hökfelt et al., 1978; Alm et al., 1980; Larsen et al., 1981; Erspamer et al., 1981; Islam et al., 1983). These are vasoactive intestinal polypeptide (VIP), substance P, somatostatin, enkephaline, and the newly discovered member of the pancreatic polypeptide family, neuropeptide Y (NPY). Of these VIP and NPY seem to be quantitatively important in smooth muscle of the lower urinary tract (Islam et al., 1983; Mattiasson et al., 1984). In the rat urinary bladder, VIP- and NPY-containing nerve fibers to a considerable extent seem to take their origin in non-adrenergic cells of the pelvic ganglia (Mattiasson et al., 1984). A certain population of the NPY-containing neurons, i.e., those around blood vessels and in the submucosa are probably to be referred to the adrenergic innervation.

Only few functional data on the effects of the neuropeptides in the lower urinary tract have hitherto been presented. Because of its pattern of distribution in the lower urinary tract, VIP has been suggested to play a role in the relaxation of smooth muscle in the bladder outlet region and the urethra (Alm et al., 1980). The effects of VIP has been investigated in detrusor muscle preparations in vitro and both contractant and relaxant effects were suggested (Johns, 1979; Larsen et al., 1981; Elmér, 1982; Klarskov et al., 1983). A clear cut role for VIP in the lower urinary tract has not been established. Even less is known about the functional significance of substance P, somatostatin and enkephaline. No functional data on the role of NPY in the lower urinary tract have as yet been presented.

Parasympathetic decentralization

In different investigations stimulation of the pelvic nerves produced increase, decrease, no change or a variable response of the intraurethral pressure (Elliot, 1906; Graber & Tanagho, 1975; Creed & Tulloch, 1978; Slack & Downie, 1983). This variety of responses might indicate a complex innervation of the urethra mediated by the parasympathetic, pelvic nerves.

Secondary to lesions of the parasympathetic innervation to the lower urinary tract, so called lower motor neuron lesions, a disturbance of the function of both the bladder and the urethra is seen. Since the functional disorder has more obvious consequences for the bladder than the urethra, the condition is referred to as autonomous bladder in clinical practice. The etiology of such a dysfunctional state can be closure defects of the spinal canal, e.g., myelomeningocele and conditions caused by trauma or other lumen-reducing processes such as herniated intervertebral discs, tumours and bleedings. Not seldom this type of dysfunction is seen after pelvic surgery.

The disturbance of the bladder function is characterized by the loss of a centrally coordinated detrusor contraction, by detrusor-hypertrophy, sprouting of adrenergic nerve terminals (Sundin & Dahlström, 1973) and an increased

contractant α -adrenergic influence (Norlén et al., 1976; Sundin et al., 1977) and supersensitivity of the detrusor to cholinceptor stimulating agents (Lapides, 1962; Glahn, 1970). In the human urethra there seems to be a decreased intraurethral pressure at rest (Sunder et al., 1978; Nordling, 1983) and also an inability of relaxation, i.e., a lack of further pressure reduction at attempts of bladder emptying (Koyanagi et al., 1982).

The inability to empty the bladder completely leads to residual urine and will thereby jeopardize the integrity of the upper urinary tract and the kidney function. The patient most often experiences the dysfunction both as an inability to achieve complete bladder emptying and as urinary incontinence.

The possibility to further reduce the urethral pressure in this group of patients by means of α -adrenoceptor blocking agents such as phentolamine makes it probable that the remaining urethral pressure after a complete lesion of the parasympathetic innervation is the result of adrenergic influence (Clarke & Thomas, 1981).

It has, however, been claimed by different investigators that the infravesical outflow obstruction in these patients would depend on a supersensitivity to α -adrenoceptor stimulation (Koyanagi, 1978; Sunder et al., 1978) similar to the supersensitivity to cholinceptorstimulation found in patients with parasympathetically decentralized bladders. It has not been shown that the urethral pressure increase measured and taken as an expression for supersensitivity is based on what can be called true supersensitivity, i.e. supersensitivity in a pharmacological sense (Westfall, 1981).

The aims of the present study were to investigate:

1. the excitatory, contractant and the inhibitory, relaxant mechanisms of the muscle layers and of the submucosa (lamina propria) in the urethral wall of the female rabbit urethra, and in muscle preparations from the sphincteric zones representing the maximum urethral pressure of the human male and female urethra.
2. the possible presence of an interaction between adrenergic and cholinergic nerves via prejunctionally located receptors in the urethra of rabbit and man.
3. the receptor basis for a possible autoregulatory influence on the transmitter release from adrenergic nerves in the urethral muscle of rabbit and man.
4. the effects of the putative neurotransmitter vasoactive intestinal polypeptide (VIP) in the bladder and urethra of rabbit and man.
5. a) the receptor-basis for possible urethral supersensitivity to α -adrenoceptor stimulating agents b) the therapeutic use of a selective α_1 -adrenoceptor blocking agent, prazosin, in the presence of infravesical outflow obstruction in patients with parasympathetic decentralization of the lower urinary tract.
6. the receptor-basis for supersensitivity of the detrusor to cholinceptor stimulating agents following parasympathetic decentralization of the lower urinary tract in an animal model.

Material and methods

In vitro investigations

Dissection and preparation

Human preparations

The human preparations were taken from the zones representing the maximum urethral pressure, i.e., the middle third of the female urethra and the infra-collicular-membraneous part of the male urethra. In the investigations of the release of ^3H -NA, preparations were also taken from the prostatic part of the urethra. The majority of preparations were obtained from patients undergoing cystourethrectomy because of bladder malignancy and who also in most cases had been treated preoperatively with irradiation against the bladder. All muscle preparations were dissected out as circularly oriented preparations from the muscle layer beneath the mucosal-submucosal layers in both the female and the male human urethra. The detrusor strip preparations were taken from the dome of the bladder.

The human urethral preparations were used both the day of surgery and the following day. The preparations not immediately used were stored at $+4^\circ\text{C}$ until used. This procedure was motivated by the restricted supply of tissue.

Rabbit preparations

Different parts of the female rabbit urethra were used. All preparations were taken from the proximal two thirds of the urethra, i.e. the narrowest part of the organ as judged in situ, which also corresponds to the zone of the maximum urethral pressure (unpublished observation). For the basal studies on the electrically induced contractant and relaxant responses (I, II, IV) ringformed preparations of the whole urethral wall were taken, each representing a length of 4 mm of the urethra.

In the functional investigations whole wall-preparations were used as well as the submucosal layer. In the investigations of the submucosal tissue, preparations were taken in the longitudinal direction, mainly from the structure comprising the urethral crest. The detrusor muscle preparations were taken from the dome of the bladder. All rabbit preparations were investigated the day the animals were sacrificed.

Treatment of rabbits with 6-hydroxydopamine (6-OHDA)

The contractant and relaxant responses of rabbit urethral preparations to electrical field stimulation were also studied after chemical sympathectomy of rabbits with 6-OHDA. The drug was dissolved in saline solution on ice during continuous bubbling with nitrogen to avoid oxidation. A dose of 70 mg/kg was then immediately given i.v. and after 24 hours the animals were sacrificed for investigation.

Functional investigations

The rabbit urethral preparations were suspended either as ring or strip-preparations between hooks in organ baths with a volume of 5 or 10 ml. The human urethral strip-preparations were investigated in the same way. The organ baths contained Krebs solution kept at 37°C , bubbled with a mixture of 95% O_2 and

5% CO₂ to give a pH of approximately 7.4. The tension of the preparations was adjusted during an equilibration period of approximately 1 h to a final level of 4–10 mN. The "isometric" tension was recorded on a Grass model 7D recorder. Drugs added to the bath did not exceed 1% of the organ bath volume except when cumulative addition was used.

Electrical field stimulation

Electrical field stimulation was performed by means of two electrodes close to and parallel with the strip preparations or, in the ring preparations, with one electrode within the lumen and the other outside, and close to the preparation. A minimum of three responses with an internal variation <10% was accepted as reproducible. The voltage chosen was supramaximum and the frequency giving a response amounting to 70–80% of maximum was usually used. Single square wave pulses with a duration of 0.8 ms were delivered in trains of 5 s with an interval of 2–4 minutes by means of a Grass S88 electrical stimulator. The polarity of the electrodes was changed after each pulse by means of a polarity changing unit.

Release studies

Human and rabbit urethral muscle preparations weighing 15–50 mg were cut into four interconnecting strips and incubated with ³H-NA (2.7 or 14.2 Ci/mmol, New England Nuclear) for 45 minutes. After rinsing in Krebs solution the preparations were placed in perfusion chambers and stimulated electrically at a frequency of 10 or 5 Hz in trains of 60 s four times in each experiment (S_I–S_{IV}). The electrically induced release of ³H at each stimulation was expressed as a fraction of the total amount of ³H in the preparation at the time of onset of stimulation. The influence of different drugs was investigated and the amount of electrically released ³H was expressed in relation to the expected size of the fractional release in a control series of experiments. In the experiments with ³H-NA the Krebs solution contained 0.3 mM ascorbic acid to stabilize the NA in the solution. In the series of experiments when the influence of VIP on the release of ³H-NA was investigated the Krebs solution contained 0.1% bovine serum albumine in order to reduce adhesion of the peptide to the walls of the experimental equipment.

Measurement of noradrenaline

In the studies on release of ³H-NA the amount of ³H-NA in the rabbit urethral preparations and in the perfusion fluid during basal rinsing conditions and after electrical stimulation was measured by means of high pressure liquid chromatography (HPLC) with electrochemical detection essentially as described by Eriksson and Persson (1982).

Light and electron microscopy

Rabbit urethral preparations representing the whole wall were fixed in glutaraldehyde and processed for light microscopy. Preparations already used in functional investigations were also processed for light microscopy in order to verify that the tissue investigated was the intended one. The preparations used for electron microscopy were after fixation in glutaraldehyde post-fixed in osmium-tetroxide (Os O₄), embedded in Araldite and cut with glass knife for subsequent investigation in a Zeiss electron microscope (×2000).

In vivo investigations

Selection of patients

The patients with lower motor neuron lesions (parasympathetic decentralization) of the lower urinary tract were chosen for participation in the investigations on the basis of clinical and urodynamic data. Patients with respiratory or cardiovascular diseases or patients on drugs which might interfere with the function of the lower urinary tract were not accepted for participation in the study. Permission for the investigations was given by the Ethics Committee of the University of Lund and the subjects investigated were fully informed and gave their written informed consent before the investigation.

Urethral pressure profiles

The urethral pressure profiles in all subjects were recorded by means of a Ch 7 double microtip transducer (Gaeltec). The three o'clock position was chosen to avoid variation of the profile with rotation in the urethra (Anderson et al., 1983).

Surgical procedures

In the investigations on the parasympathetically decentralized feline urinary bladder decentralization was performed by means of division of the ventral sacral roots S_I-S_{III} . Silverclips were applied on the proximal root endings to prevent regeneration. Postoperatively the bladders were emptied passively.

Urinary diversion was performed, using microsurgical technique, by means of bilateral uretero-sigmoideo-cutaneostomy. In the group of animals subjected to both urinary diversion and parasympathetic decentralization the diversion preceded the decentralization with 3 weeks. The animals were sacrificed ten to twelve weeks after the last operation and preparations were taken in a standardized manner from both bladder halves for investigations. The sensitivity to carbachol was investigated by means of cumulative addition of the drug. The mechanical investigations are described in (VIII). The experiments were approved by the Animal Ethics Committee of the University of Lund.

Statistical analysis

Statistical analysis was performed using the Student's test for paired and unpaired data, and also analysis of variance. A probability level less than 5% was regarded as significant.

RESULTS

I Adrenergic and non-adrenergic contraction of isolated urethral muscle from rabbit and man.

Rabbit

The contractant response to electrical stimulation consisted of two main components (Fig 1), one fast non-adrenergic, non-cholinergic and one slow, adrenergic. The adrenergic component usually consisted of two phases, the first of which was of a higher amplitude than the second. The first phase of the adrenergic component seemed to be mediated mainly via α_1 - and the second mainly via α_2 -adrenoceptors. A certain increase of the amplitude of the adrenergic component was observed in the presence of ACh while a decrease of the response was seen in the presence of scopolamine.

The adrenergic component disappeared completely after treatment of rabbits with 6-OHDA, while the fast, non-adrenergic, contractant component did not seem to be affected by this treatment. The fast component lasted only during the stimulation period and was not sensitive to adreno- or cholinceptor blocking agents or to curare. The response was however readily blocked by tetrodotoxin 10^{-6} M. After removal of the longitudinal muscle layer no change in the appearance of the contractant complex was seen.

The two main components could be separated from each other by lowering the temperature of the organ bath from 37 to 20°C and such a separation was also observed in some preparations at 37°C. Another difference between the two main components was a strong dependence on the presence of Ca^{++} for the adrenergic response, while the non-adrenergic component was more resistant to Ca^{++} -depletion.

The amplitude of the non-adrenergic component depended on the tension of the preparation, i.e. after contraction by means of, e.g. NA the non-adrenergic response increased considerably in size (Fig 1).

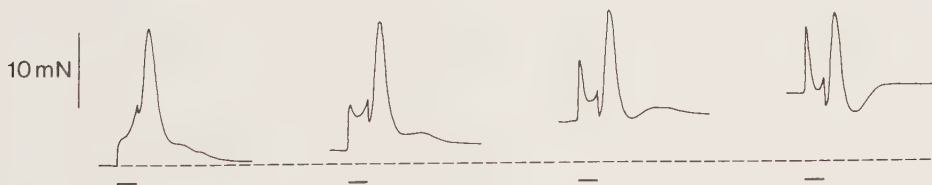


Fig. 1 The response of an isolated rabbit urethral ring preparation to electrical field stimulation. The influence of increasing the "basal tension level" by means of noradrenaline is shown. Bars represent stimulation trains = 5 s.

Man

Prazosin (selective for α_1 -adrenoceptors) but not raulwolscine (selective for α_2 -adrenoceptors) was found to be a potent inhibitor of the NA-induced contraction in the human urethra. In preparations from only one patient out of eight investigated, the α_2 -adrenoceptor agonist clonidine induced a contractant response, amounting to 8% of the response induced by NA.

At electrical field stimulation of the human urethral preparations a fast non-adrenergic response was seen as described for the rabbit urethral preparations (Fig 2). The different responses were usually not seen in the same preparation. The sensitivity of the slower contractant components to tetrodotoxin was less pronounced than in the rabbit. The adrenergic component seemed to be mediated via α_1 -adrenoceptors. Clonidine reduced or abolished the adrenergic component. Scopolamine in a concentration of 10^{-5} M reduced the slower contractant response by $39 \pm 6\%$ in six preparations while no effect was seen in another three preparations.

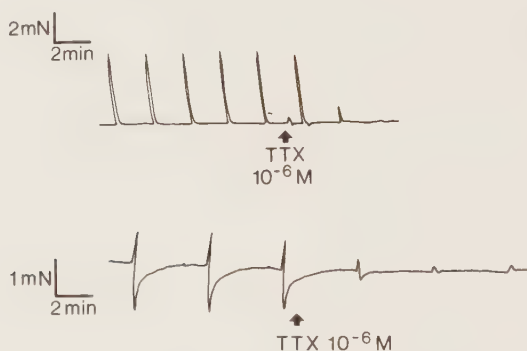


Fig. 2 Electrically induced responses of isolated human female (upper curve) and male (lower curve) urethral preparations, taken from the zones representing the maximum urethral pressure. The preparations were exposed to prazosin 10^{-6} M, rauwolscine 10^{-6} M and atropine 10^{-5} M (female preparation), phentolamine 10^{-6} M and scopolamine 10^{-5} M (male preparation) with contact times of 15 minutes at the time of recording. TTX=tetrodotoxin. Stimulation frequency 15 Hz.

Light microscopy

The light microscopic investigation of the female rabbit urethra confirmed the presence of an outer longitudinal and an inner circular muscle layer. The cells of the circular layer showed smooth muscle characteristics. This layer was most prominent in the middle third of the urethra. The longitudinal layer was thickest in the proximal part of the urethra, but like the circular layer found along the whole organ. The muscle cells of the longitudinal layer comprised two populations of cells, one with large striated cells and one with smooth muscle cells of considerably smaller size. Bundles with a striated muscle cell predominance were found but also sections through the longitudinal layer where smooth muscle cells dominated and where only scattered striated muscle cells were found.

Electron microscopy

Electron microscopy confirmed the findings of the light microscopic investigation, i.e. both smooth and striated muscle cells in the longitudinal layer and only smooth muscle cells in the circular layer and the submucosa.

II Electrically induced relaxation of the noradrenaline contracted isolated urethra from rabbit and man.

Rabbit

The preparations were contracted by means of NA and electrical field stimulation induced a pronounced, frequency-dependent, TTX-sensitive relaxant response with a threshold at 1–2 Hz and maximum at 15–20 Hz. The maximum relaxant response in the rabbit preparations amounted to $57 \pm 2\%$ of the NA-induced tension increase. At continuous electrical stimulation the response could be maintained for several minutes. After cessation of stimulation the tension of the preparations returned to the original level, which also was seen at exposure to TTX during continuous stimulation (Fig. 3). The magnitude of the relaxant response was not influenced by adreno- or cholinergic active drugs. It could neither be mimicked nor blocked by any of a variety of drugs known to be active at autonomic receptors. Isoprenaline, adenosine, ATP, PGE_2 and VIP produced slowly developing and incomplete relaxation of the rabbit urethral preparations.

Also when contraction of rabbit urethral preparations was induced with clonidine, lysine vasopressin and 5-hydroxytryptamine electrical stimulation evoked a relaxant response with the same appearance as after contraction with NA. In a separate series of experiments on rabbit urethral preparations from animals treated in vivo with 6-OHDA to achieve sympathectomy, the relaxant response was still demonstrable with an appearance of that in NA-contracted preparations from animals not subjected to 6-OHDA-treatment.

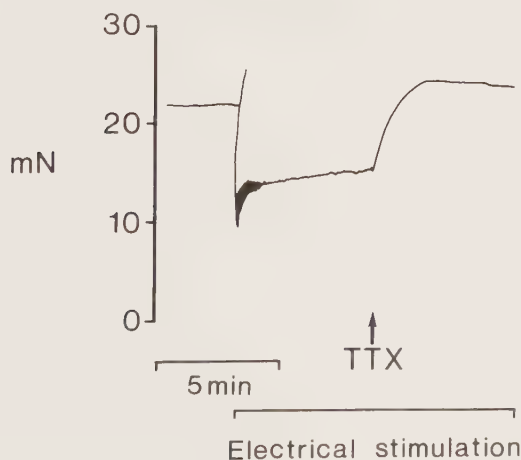


Fig. 3 The influence of continuous electrical stimulation on an isolated female rabbit urethral ring preparation contracted by means of noradrenaline ($6 \times 10^{-5} \text{ M}$). Addition of tetrodotoxin (TTX) 10^{-6} M reestablishes the tension to the prestimulation level. Stimulation frequency 8 Hz.

Man

In the human urethral preparations a relaxant response of similar appearance as that seen in the rabbit was evoked by electrical stimulation (Fig. 4). However, in the preparations from the supracollicular part of the male urethra a superimposed contraction was seen.

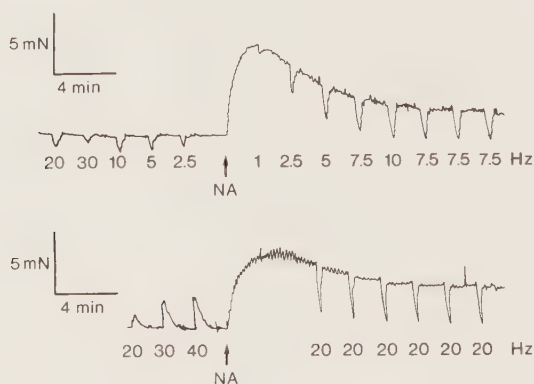


Fig. 4 The electrically induced responses at different stimulation frequencies in preparations from the infracollicular part of the male human urethra before and after contraction of the preparations by means of noradrenaline (NA) $6 \times 10^{-5} M$.

III Adrenoceptors and cholinoceptors controlling the release of noradrenaline from adrenergic nerves in the isolated urethra from rabbit and man.

After preincubation with 3H -NA rabbit urethral preparations containing circular and longitudinal muscle layers and human urethral muscle preparations were placed in perfusion chambers and subjected to intermittent electrical field stimulation. The electrically induced release of 3H from the adrenergic nerves was well reproducible. It was abolished in the presence of TTX. The amount of electrically induced release of 3H from rabbit urethral preparations was shown to consist to 47% of 3H -NA, while the amount of 3H -NA in the preparations comprised 91% of the total amount of 3H . The release of 3H was decreased in the presence of clonidine, and increased after exposure to the α_2 -adrenoceptor blocker rauwolscine (Fig. 5a). The muscarinic cholinoceptor stimulating and blocking agents carbachol and scopolamine (and atropine) decreased respectively increased the electrically induced release of 3H (Fig. 5b). No qualitative differences in the responses to α_2 -adrenoceptor or muscarinic cholinoceptor active agents were seen in the rabbit compared to the human urethral preparations.

β -adrenoceptor stimulation produced a certain, but insignificant, increase of the release, while β -receptor blockade by means of propranolol induced a significant decrease of the release of 3H . The presence of α_1 -adrenoceptors or nicotinic cholinoceptors could not be demonstrated.

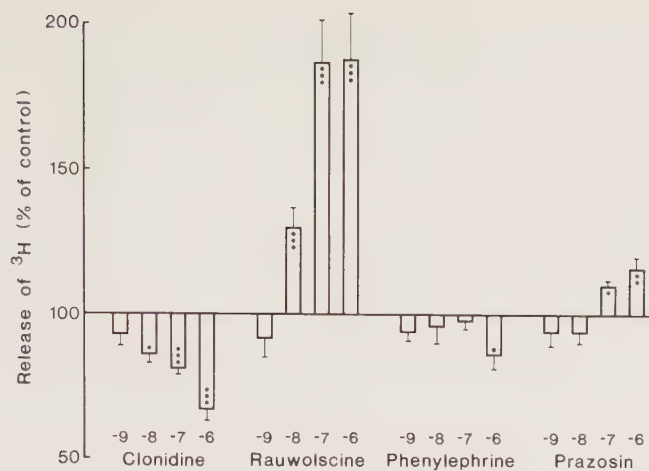
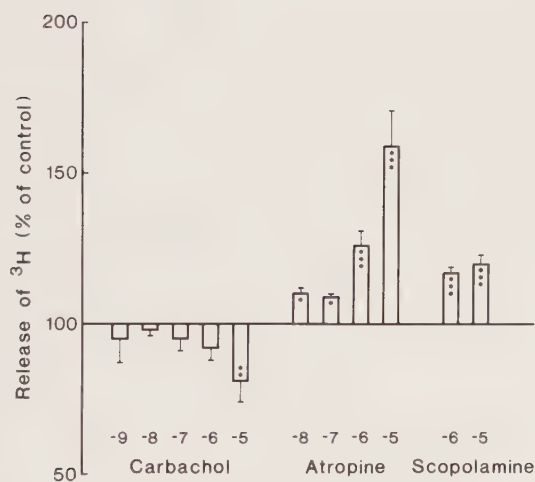
a**b**

Fig. 5a+b Changes of the electrically induced efflux of ^3H from female rabbit urethral preparations in the presence of α -adrenoceptor and muscarinic cholinergic stimulating and blocking agents. The results are expressed in % of a series of control experiments ($n=12$). Mean $\pm$ SEM are given. $n=6-8$, $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***

IV Effects of vasoactive intestinal polypeptide on isolated urethral and urinary bladder smooth muscle from rabbit and man.

Urethra

VIP was found to inhibit the contractions induced by submaximum concentrations of NA in rabbit urethral preparations by maximum $71 \pm 5\%$ (Fig. 6a). The inhibition of electrically induced contractions produced by VIP $5 \times 10^{-7} \text{ M}$ was $52 \pm 4\%$ and $59 \pm 7\%$ when, respectively, a low (7–10 Hz) and high (25–30 Hz) frequency of stimulation was used (Fig. 6a). The inhibitory effect on the

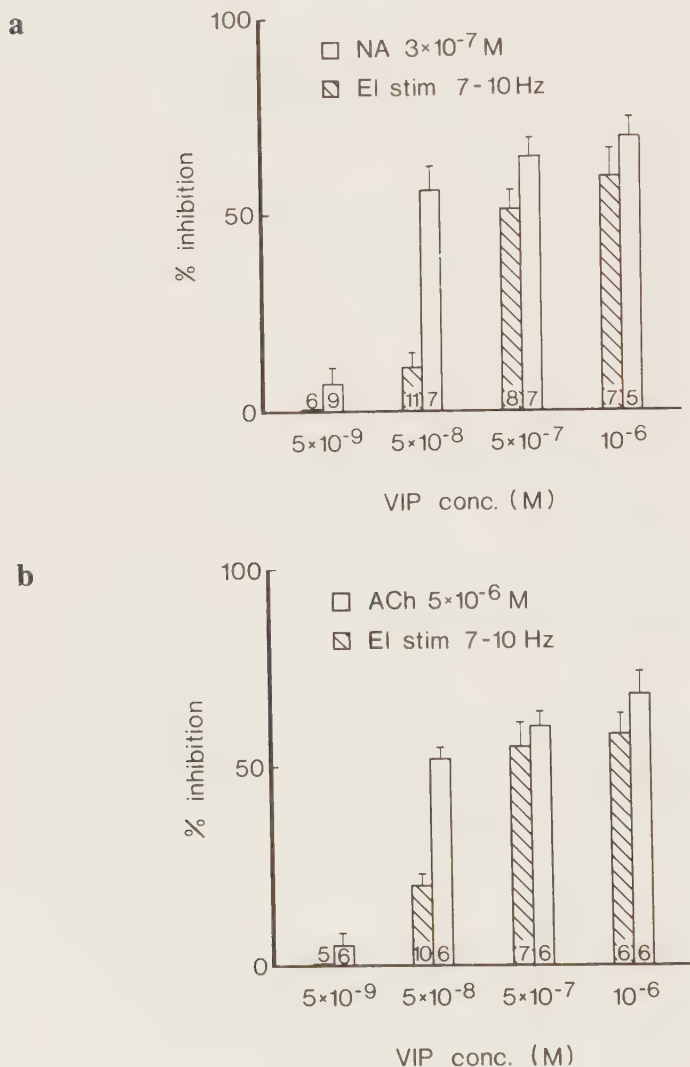


Fig. 6a+b The inhibitory effect of vasoactive intestinal polypeptide (VIP) on contractant responses of female rabbit urethral ring (a) and bladder strip (b) preparations (mean $\pm$ SEM). Contractions were induced by exogenous application of noradrenaline (NA $3 \times 10^{-7} \text{ M}$, urethral preparations), acetylcholine (ACh $5 \times 10^{-6} \text{ M}$, bladder preparations) and submaximum frequency (7–10 Hz) electrical field stimulation. The number of experiments are given within the bars.

adrenergic component of the contraction was approximately the same of phenolamine and VIP. In the human urethral preparations VIP 5×10^{-7} M was found to inhibit the NA-induced contraction by $29 \pm 9\%$ (range 0–85%). No consistent effect were found on the electrically induced contractant response of the human urethra.

Bladder

VIP was found to inhibit the contractile response to submaximum concentrations of ACh (5×10^{-6} M and 5×10^{-5} M) in rabbit detrusor strips by maximum $68 \pm 6\%$ (Fig. 6b).

The electrically evoked contractant response in these preparations was inhibited by maximum $58 \pm 6\%$ (Fig. 6b). There was no significant difference in the blocking potency of VIP at high and low frequencies of stimulation. Isoprenaline 10^{-6} M inhibited the electrically induced contractant response in the rabbit bladder by $70 \pm 5\%$. Pretreatment with propranolol did not change the inhibitory effect of VIP, but blocked the inhibitory effect induced by isoprenaline.

In atropine or scopolamine-treated preparations a contractant response amounting to 27% of the originally remained, i.e. the non-cholinergic (atropineresistant) component. This part of the contractant response was inhibited by VIP 5×10^{-7} M by $93 \pm 2\%$.

No effects of VIP were found on ACh-induced contractions or on the electrically induced contractant response of the human detrusor.

The possible effects of VIP on the release of NA from adrenergic neurons was also investigated. In the model used, VIP 5×10^{-7} M had no effects on the electrically induced release of ^3H from preparations preincubated with ^3H -NA.

V Contractant and relaxant properties of the female rabbit urethral submucosa

Microscopic investigation revealed an abundant occurrence of smooth muscle cells within the submucosa. The muscle cells formed longitudinally oriented bundles in the depth of this layer of the urethral wall, close to the well defined circular muscle layer. Light microscopic investigation confirmed that submucosal tissue was investigated.

NA-induced contractions of the preparations and the contractant response to electrical field stimulation (Fig. 7) was blocked by α -adrenoceptor blocking

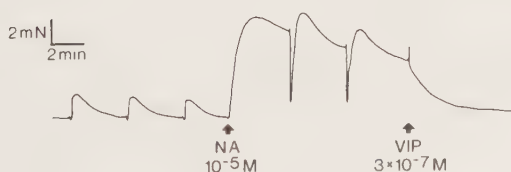


Fig. 7 The responses of a submucosal preparation from the female rabbit urethra to electrical stimulation (frequency 25 Hz) at basal conditions and after contraction of the preparation induced by noradrenaline (NA). After the second relaxant response electrical stimulation was stopped. Addition of vasoactive intestinal polypeptide (VIP) induced a complete relaxation of the preparation.

agents and by TTX. The α_2 -adrenoceptor blocking agent rauwolfscine was a more potent blocker than the α_1 -adrenoceptor blocking agent prazosin. ACh did not induce contraction, and scopolamine had almost no effect on the electrically induced response. When the preparations were contracted by a submaximum concentration of NA a relaxant response amounting to $35 \pm 6\%$ was induced by a high concentration of ACh, 10^{-4}M . The neuropeptides VIP and NPY inhibited the contractant response to electrical field stimulation. VIP was also a potent blocker of contractions induced by exogenously applied NA. NPY was a less potent blocker of the nerve-mediated response and induced also a certain contraction of the preparation. When the preparations were contracted by means of NA the response to electrical field stimulation was no longer contraction, but relaxation. This response was pronounced and amounted to $82 \pm 1\%$ of the NA-induced contraction and was significantly ($p < 0.05$) more pronounced after contraction of the same preparations to a comparable tension level by means of clonidine.

VI Urethral sensitivity to α -adrenoceptor stimulation and blockade in patients with parasympathetically decentralized lower urinary tract and in healthy volunteers.

A greater urethral pressure increase has been observed after administration of a single dose or concentration of an α -adrenoceptor stimulating agent in patients with neurogenic lesions of the lower urinary tract, e.g. after parasympathetic decentralization, than in normal subjects. This has been interpreted as supersensitivity. No pharmacologically based evidence for supersensitivity has been presented.

In this study NA ($4\mu\text{g/ml}$) was given as a continuous infusion in step-wise increased doses to patients with parasympathetic decentralization of the lower urinary tract and to healthy volunteers. The urethral pressure in the patient group increased more in absolute terms than in the control group, but expressed as a dose-response relation no differences in sensitivity to NA were found between the two groups (Fig. 8a, b). An initial decrease of the maximum urethral pressure was seen in the group of female volunteers, while an increase was seen in the other groups. The decrease of the urethral pressure induced by phentolamine was greater in the group of normal females than in normal men. The reduction of the maximum urethral closure pressure induced by phentolamine was most pronounced in the patient group. The concentrations of NA/s were measured in eight of the healthy volunteers and found to be almost linearly related to the infusion rate of NA. There were no differences in blood pressure increase between patients and healthy volunteers.

VII Effects of prazosin on isolated human urethra and in patients with lower motor neuron lesions.

In vitro

The selective α_1 -adrenoceptor blocking agent prazosin and the non-selective α -adrenoceptor blocker phentolamine were in vitro shown to block NA-induced contractions of the isolated human urethral muscle in a concentration-dependent way. Prazosin was on a molar basis approximately ten times more effective than phentolamine. Preparations contracted by NA could be completely relaxed by prazosin.

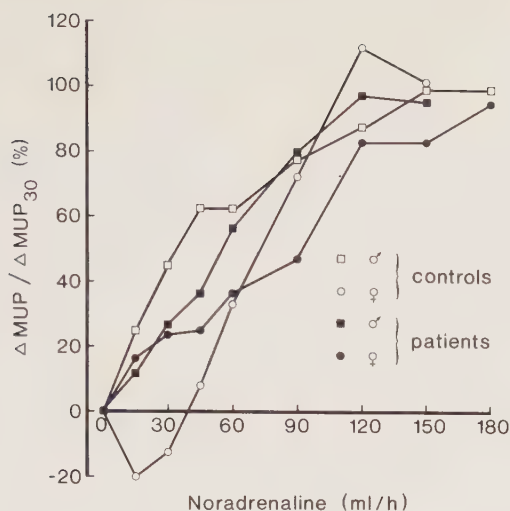
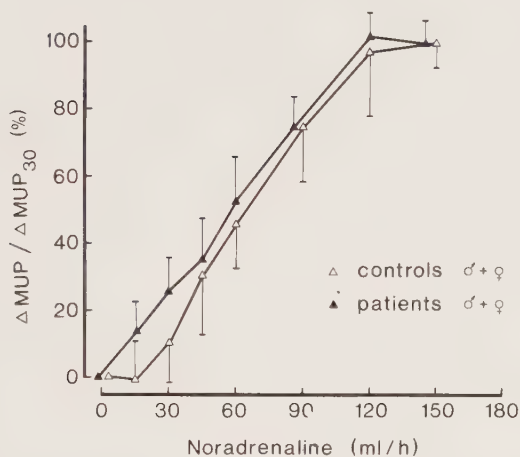
a**b**

Fig. 8a+b The percentual increase of the maximum urethral pressure (MUP) in the interval $MUP_0 - MUP_{30}$ (for explanation, see VI) induced by i.v. infusion of noradrenaline $4\mu\text{g/ml}$, in patients with parasympathetic decentralization of the lower urinary tract and in healthy volunteers. Bars indicate SEM.

In vivo

Seven patients with lower motor neuron lesions, i.e. parasympathetic decentralization of the lower urinary tract, and with poor bladder emptying, were treated with prazosin p.o. 2 mg b.i.d. for more than two weeks. Five of the patients experienced a considerable improvement of voiding ability and continence compared to the situation before treatment. Five patients were urodynamically investigated before and during the period of treatment and in these a reduction of the intraurethral pressure and the bladder pressure during filling was seen and in two patients also an inhibition of "autonomous bladder waves". The only side effect noted was one case of nasal congestion.

VIII Supersensitivity to carbachol in the parasympathetically decentralized feline urinary bladder.

The receptor-basis for the increased sensitivity to cholinceptorstimulating agents after parasympathetic decentralization was investigated in an experimental study on the feline urinary bladder. Detrusor hypertrophy with a 4–5-fold increase of the bladder weight (mainly muscle mass) was found after parasympathetic decentralization but not when urinary diversion preceded the decentralization and not after urinary diversion only. The light microscopic investigation revealed an increased density of smooth muscle bundles in the detrusor strips from bladders subjected to parasympathetic decentralization only. The size of the cross-sectioned cells in this group was considerably greater than in the other groups investigated.

The sensitivity to carbachol expressed as EC_{50} was significantly increased after decentralization, but only in the hypertrophic bladders (Fig. 9). A small and non-significant difference in the slope factors of the concentration response curves to carbachol in the four groups was seen. The ability to production of active force was decreased in all the operated groups to approximately 50% of that in the control group. No differences in the passive mechanical properties were found between the hypertrophic and the normal bladders.

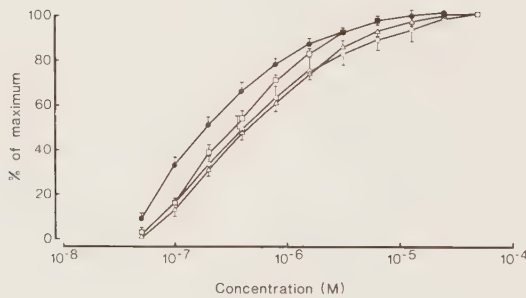


Fig. 9 Carbachol concentration-response curves for detrusor preparations from normal cats ($n=4$, ○) and from cats subjected to parasympathetic decentralization ($n=6$, ●), urinary diversion only ($n=2$, △) and urinary diversion followed by parasympathetic decentralization ($n=4$, □). The number of preparations investigated = 14–31.

General discussion

The urethra

Adrenoceptor mediated functions

α -Adrenoceptor mediated contraction in the urethral smooth muscle has previously been demonstrated in vivo and in vitro in different animal species and in man (Donker et al., 1972; Awad & Downie, 1976). Not only the well defined circular muscle layer of the rabbit urethra was found to contract in response to α -adrenoceptor stimulation, but also the submucosal tissue. In addition, this tissue exhibited relaxant properties (V). In the female rabbit urethra it was found that both subtypes of α -adrenoceptor, α_1 and α_2 , mediated contractile responses (Andersson et al., 1984). In the present study evidence was obtained that the contraction mediating, postjunctional α -adrenoceptors in the human urethra are of α_1 -type (I, VII). Prejunctional α_2 -adrenoceptors mediating inhibition of NA release were found in the female rabbit urethra, as well as in human urethral preparations (III). The present results from rabbit urethra showed that the distribution of the different α -adrenoceptor subtypes postjunctionally was not the same in different parts of the rabbit urethral wall (I, V). In the submucosal tissue, α_2 -adrenoceptors seemed to mediate the major part of the contractant response to exogenously applied NA and of the contraction induced by electrical stimulation (V), while in the muscle layers the α_1 -adrenoceptors were of greater importance (I). It may be speculated that this difference in receptor distribution within the urethral wall was also reflected by the configuration of the contractant response to electrical field stimulation in preparations containing all layers of the urethral wall. The initial adrenergic, and mainly α_1 -adrenoceptor-mediated, part of the response in the ring preparations may thus represent the circular muscle layer, while the slower component, which was more sensitive to blockade with rauwolscine, the contractant response of the submucosal layer.

It might be that α_1 -adrenoceptors are located mainly intrajunctionally, while α_2 -adrenoceptors have an extrajunctional localization (Langer & Shepperson, 1982). If this is valid also for the urethra it might imply important differences between the functions of the two types of adrenoceptor. This is especially interesting in view of the finding that the number of α_2 -, but not α_1 -adrenoceptors increase significantly in the female rabbit urethra after systemic administration of estrogen (Larsson et al., 1984).

Prejunctional α_2 -adrenoceptors on adrenergic nerve-endings (III), mediating a negative feed-back on the release of NA has previously been demonstrated in other tissues with adrenergic innervation (Starke, 1977; Langer, 1981). These receptors might provide a way to influence the sympathetic nervous activity by means of pharmacological tools. It cannot be excluded that the decrease of the urethral pressure after administration of clonidine to patients with minor urologic complaints observed by Nordling et al. (1979) and to patients with neurogenic lesions (Rao et al., 1980) was mediated via prejunctional α_2 -adrenoceptors on adrenergic urethral nerve terminals in addition to an effect on the central nervous system.

Cholinoceptor mediated functions

The cholinergic innervation of the urethra is relatively rich (Elbadawi, 1982) and the amount of muscarinic cholinoceptors in the rabbit urethral muscle was

found to be approximately 2/3 of that in the detrusor (Johns, 1983). The rabbit urethral ring preparations did not respond with contraction on exposure to ACh which should mean that contraction of the longitudinal muscle layer did not significantly contribute to the response (I).

The influence of muscarinic cholinceptor blocking agents on the electrically induced contractions of the isolated human urethral smooth muscle preparations (I) indicates that the relatively rich cholinergic innervation is of importance for the control also of human urethral smooth muscle. However, circular preparations of human urethral smooth muscle do not contract when exposed to cholinceptor stimulating agents (Ek et al., 1977) and in human subjects the maximum urethral pressure at rest is not changed after administration of cholinceptor stimulating or blocking drugs (Ulmsten & Andersson 1977. Ek et al., 1978).

The discrepancy between the effects of muscarinic cholinceptor stimulation found in the functional investigations (I) and the ^3H -NA release studies (III), i.e. an increase of the α -adrenoceptor mediated contractant response to electrical stimulation and a decrease of the release of ^3H -NA in the presence of ACh (carbachol) is difficult to explain. It might reflect the complexity of effects pre- and postjunctionally induced by the agents released at electrical field stimulation.

Adrenergic-cholinergic interaction

An interaction between peripheral adrenergic and cholinergic nerve fibers in the lower urinary tract has on morphological grounds been suggested by Elbadawi and Schenk (1974). In the present study the receptor basis for such an interaction was demonstrated in urethral muscle preparations from both rabbit and man (III).

In whole wall rabbit urethral preparations and in human urethral muscle preparations prejunctional muscarinic cholinceptors on the adrenergic nerve endings were shown to inhibit the release of NA. This means that an increased parasympathetic activity, e.g., at the initiation of micturition may cause a decrease of intraurethral pressure by inhibition of sympathetically mediated urethral tone (see below).

Non-adrenergic, non-cholinergic functions

Contraction

The fast part of the contraction induced by electrical stimulation and observed in rabbit as well as in human urethral muscle preparations (I), seems to be mediated by a non-adrenergic, non-cholinergic transmitter released from nerves. This view is supported by the results of the experiments showing that after removal of the longitudinal muscle layer, the response could still be demonstrated. A significant reduction was seen after removal of the mucosal and submucosal tissue (unpublished). However, when the different layers of the urethral wall were investigated separately the fast contractant component was no longer observed. No explanation to this finding can presently be offered. Whether this fast response is an expression for activity mediated in the parasympathetic, pelvic nerves or if it is an expression for the presence of another type of nervous activity remains to be established. Interestingly, a fast and twitch-like activation of the feline urethra in response to hypogastric nerve stimulation has recently been demonstrated (Slack & Downie, 1983).

It can not be excluded that the fast contractant component demonstrated in the present investigation may be of importance also in vivo. It might be speculated that it comprises a part of the active mechanism producing urethral closure during stress.

Relaxation

Inhibition of the contractile forces contributing to the closure of the urethra, producing a decrease of the intraurethral pressure, might be expected during the emptying phase of the bladder. A decrease of the intraurethral pressure during micturition, preceding the detrusor contraction has been demonstrated by several investigators (e.g. Tanagho and Miller 1970). Such inhibitory activity or decrease of active tension can be mediated in several ways, e.g., a reduced frequency of excitatory nerve pulses, an inhibition of the release of excitatory transmitter(s) at the nerve terminal level, and release of a relaxation-mediating transmitter. The presence of a relaxation-mediating transmitter in the urethra of rabbit and man was suggested by the results of the present investigation (II,V). The site of action of this transmitter was most probably postjunctional. The presence of a non-adrenergic, non-cholinergic transmitter in the urethra has been suggested also by Slack and Downie (1983), who demonstrated a decreased resistance to flow in the feline urethra during ventral sacral root stimulation and by Klarskov et al., (1983) who in vitro studied lower urinary tract smooth muscle of pig and man.

In the rabbit urethra the relaxant response was found in both whole wall urethral preparations (II) and in the submucosa (V). It was more pronounced in the submucosa than in the whole wall preparations and significantly more pronounced when the preparations were contracted by clonidine than by NA. α_2 -adrenoceptors are considered to be more dependent on extracellular Ca^{++} than α_1 -adrenoceptors (van Meel et al., 1981) Whether the results reflect that the unknown transmitter acts by interfering with calcium influx can only be speculated upon.

It can not be excluded that the observed response is of a physiological importance, and that it contributes to the decrease of the urethral pressure seen before the detrusor starts contracting at micturition. If this is true, it might be that the "urethral instability" (McGuire, 1978) sometimes seen together with uninhibited detrusor contractions (Hindmarsch et al., 1983) is due to an uncontrolled release of a relaxation-mediating transmitter.

Neuropeptides

VIP

In the present investigation (IV, V) VIP was found to be a potent inhibitor of the α -adrenoceptor mediated contractant response of the rabbit urethra and of the muscarinic cholinergic mediated contraction as well as the non-adrenergic, non-cholinergic contractant response of the rabbit urinary bladder. The effects of VIP on human urethral preparations (IV) were usually small and only in a few preparations an inhibition of 50% or more of the NA-induced contraction was seen. In the human bladder preparations, no effects of VIP were found on contractant responses induced by ACh or electrical field stimulation (IV). This finding does not support the view that VIP should act to inhibit contraction of the detrusor during the filling phase of the bladder (Gu et al., 1983).

From the present data it cannot be excluded that VIP is a relaxation-mediating

transmitter in the lower urinary tract of the rabbit. Whether VIP is the unknown transmitter mediating the electrically induced relaxant response will probably not be clarified before a selective blocking agent is available. Since the effects of VIP on human urethral and bladder smooth muscle strips generally were small or absent (IV), VIP does not seem to be of the same importance in the human as in the rabbit lower urinary tract. It must, however, be kept in mind that the rabbit preparations represented the whole urethral wall, while each human preparation comprised only a small part of the urethral wall.

NPY

The effects of NPY in the submucosa (V) were similar to those described in other tissues, e.g. cerebral blood vessels (Edvinsson et al., 1983) and vas deferens (Lundberg et al., 1982), i.e. a direct contractant response and inhibition of the contraction induced by electrical field stimulation. In the lower urinary tract NPY has a rich occurrence both in rat (Mattiasson et al., 1984) and man (Islam et al., 1983). Since that antisera used in the immunocytochemical investigations of neuropeptide containing neurons almost exclusively are of the anti-rabbit type, this kind of morphological investigations on the rabbit urethra can be expected to be unrewarding. Further investigations on the distribution and effects of NPY in the urethra and bladder must be performed before any role for this peptide in the lower urinary tract can be suggested.

Parasympathetic decentralization

Based on the present *in vitro* data (I, III, V) it seems that the parasympathetic innervation might be of importance both for activation of urethral muscle and for inhibition of adrenergic activity. This view is in consonance with the findings of a decrease of the maximum urethral pressure in patients with parasympathetic decentralization (Sunder et al., 1978; Nordling et al., 1981 and VI), and not necessarily in conflict with the observation that the parasympathetic innervation of the urethra in, e.g., the cat seems to be without importance for the maintenance of the urethral smooth muscle tone at rest (McGuire & Herlihy, 1979). Also the results of nerve stimulation experiments *in vivo* indicate a role for the parasympathetic innervation in the regulation of the urethral pressure (Elliot, 1906; Graber & Tanagho, 1975; Creed & Tulloch, 1978; Slack & Downie, 1983).

The *in vivo* investigations of the present study (VI, VII, VIII) were performed in order to elucidate the role of parasympathetic decentralization of the bladder and the urethra on some receptor mediated functions of possible clinical importance.

Provided that the present findings (VIII) in the parasympathetically decentralized feline urinary bladder are valid also for man, it might be that the supersensitivity to cholinergic stimulating agents in patients with parasympathetic decentralization of the lower urinary tract, and reflected as a pressure increase in the bladder, is related more to the presence of hypertrophy than to the neurogenic lesion *per se*. This would contribute to explain that clinical supersensitivity tests are often found to be non-specific (Blaivas et al., 1980).

A pharmacological basis for urethral supersensitivity to α -adrenoceptor stimulation was not found in the present investigation (VI). This is in line with the findings of Cumes et al., (1979) in dogs. The value of the clinical test

suggested by Nordling et al. (1981) for diagnosis of lesions of the autonomic (sympathetic) innervation of the urethra might thus be questioned. It cannot be excluded that the presence of a lower than normal maximum urethral pressure together with the clinical findings, reflecting a lesion of the autonomic innervation of the lower urinary tract, provide a better tool for diagnosis.

It thus seems as if the infravesical outflow obstruction in these patients rather depend on an inability of relaxation, i.e. a lack of further pressure reduction at attempt of micturition, than on a changed sensitivity to α -adrenoceptor stimulating agents.

To achieve a better outflow in the presence of infravesical obstruction phenoxybenzamine has often been given these patients, often with several side-effects. In vitro we found α_1 -adrenoceptors to be of great importance for the post-junctionally mediated contraction of human urethral smooth muscle and based on these findings the selective α_1 -adrenoceptor blocking agent, prazosin, was tested in a group of patients with established outflow problems (VII). Prazosin was found to give as good therapeutic results as unselective blockade and had few side-effects in the small group of patients investigated.

Summary

- 1) In urethral smooth muscle from both rabbit and man, there is a non-adrenergic, non-cholinergic nerve mediated component in the contraction induced by transmural electrical stimulation.
- 2) A non-adrenergic, non-cholinergic relaxation was demonstrated in the urethral smooth muscle of both rabbit and man.
- 3) The receptor-basis for an autoregulation (negative feed-back) of the release of NA from adrenergic nerves, and for an interaction between adrenergic and cholinergic nerves at the nerve terminal level in the urethra were found in both rabbit and man.
- 4) VIP inhibited the adrenergic (urethra) and cholinergic (bladder) and non-adrenergic, non-cholinergic (bladder) contractant activity in the rabbit lower urinary tract. In the human urethra a certain inhibition of NA-induced contractions was seen, while no effects were found on human detrusor preparations.
- 5) Contraction of urethral muscle involving α -adrenoceptors was found to be mediated mainly via α_1 -adrenoceptors both in man and in rabbit (circular muscle layer).
- 6) In the female rabbit urethral submucosal tissue an α -adrenoceptor (mainly α_2) mediated contraction and a relaxation mediated via a non-adrenergic, non-cholinergic nervous mechanism were demonstrated.
- 7) NPY induced contraction and inhibited the nerve-mediated contraction of submucosal tissue.
- 8) In patients with parasympathetic decentralization of the lower urinary tract no evidence for an increased sensitivity in the urethra to α -adrenoceptor-stimulation was found.
- 9) The supersensitivity to cholinceptor-stimulating agents in the parasympathetically decentralized feline urinary bladder seemed to be related more to detrusor hypertrophy than to the neurogenic lesion per se.
- 10) The selective α_1 -adrenoceptor blocking agent prazosin was a potent inhibitor of NA-induced contractions in human urethral preparations, and effectively reduced the urethral outflow resistance in patients with parasympathetic decentralization of the lower urinary tract.

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Papers I—VIII

I

Adrenergic and Non-adrenergic Contraction of Isolated Urethral Muscle from Rabbit and Man

By

Anders Mattiasson, Karl-Erik Andersson and Christer Sjögren

From

The Departements of Clinical Pharmacology and Urology, University Hospital, Lund, Sweden and the Department of Obstetrics and Gynaecology, University of Toronto, Toronto Ontario, Canada

Abstract

Isolated urethral muscle from rabbit and man was subjected to electrical field stimulation and the components of the resulting contraction were analyzed, more detailed in rabbit muscle. The contraction induced consisted usually of two main components. One was rapidly developing, non-adrenergic and atropine-resistant, the other more slowly developing and sensitive to α -adrenoceptor blockade and to chemical sympathectomy with 6-hydroxydopamine. By lowering the temperature from 37°C to 20°C, these contraction components could be separated from each other. Both could be blocked by tetrodotoxin but the effects of this drug were not consistent in human tissue. Characteristic for the initial contraction component was its dependence on the tension of the preparation; it increased with increasing tension. The adrenergic part of the contraction could be effectively blocked by phentolamine and prazosin, whereas rauwolscine was less effective. Also atropine and scopolamine inhibited the adrenergic part of the contraction, whereas acetylcholine caused a transient increase. The non-adrenergic contraction component was less sensitive to deprivation of extracellular Ca^{2+} than the adrenergic; almost 40 per cent remained after exposure to Ca^{2+} free solution for 40 min, whereas the adrenergic component disappeared after 20 min exposure. Light and electron microscopic investigation revealed three distinct layers of the rabbit urethral wall, one outer consisting of smooth and striated muscle, one middle consisting of smooth muscle only, and a submucosal layer where vessels and smooth muscle cells were found. Removal of the longitudinal muscle layer did not change the responses to electrical stimulation. The results suggest that the electrically induced adrenergic activation of urethral muscle of both rabbit and man was mediated mainly via α_1 -adrenoceptors and that this muscle is innervated not only by sympathetic, adrenergic nerves but also by a type of nerves able to release a non-cholinergic, contraction-mediating transmitter.

Introduction

It is widely accepted that tone of urethral smooth muscle and intraurethral pressure to a considerable extent is maintained by the activity of the sympathetic nervous system. Supporting this view, Elliot¹ found that stimulation of the hypogastric nerves in cats constricted the urethra, and later investigations in man have shown that α -adrenoceptor stimulation increases and α -adrenoceptor blockade effectively lowers the intraurethral pressure²⁻⁴.

The response of the urethra to pelvic nerve stimulation has been reported as relaxation, contraction or no response^{1,5,6}. In the anaesthetized dog, Creed and Tulloch⁷ found that pelvic nerve stimulation increased the intraurethral pressure, and that this response was resistant to both α -adrenoceptor blockade (phentolamine) and to atropine. A contraction component resistant to α -adrenoceptor blockade and to atropine was found also by Sundin and Dahlström⁸ in the isolated cat urethra. However, the functional role of the parasympathetic nerves for regulation of intraurethral pressure has not been established. In patients with lesions of the parasympathetic sacral innervation of the lower urinary tract a decrease of this pressure has been observed by several investigators^{9,10}. Even if muscarinic cholinergic stimulating and blocking agents have almost no effect on the intraurethral pressure in man^{2,4,11}, it cannot be excluded that the parasympathetic nerves to the lower urinary tract contribute to the control of the intraurethral pressure.

In the present study, the possible presence of a non-adrenergic, non-cholinergic excitatory nervous influence on the isolated urethral smooth muscle of rabbit and man was investigated by means of electrical field stimulation.

Material and methods

Rabbit

Female rabbits of the Danish land race (n=94) weighing approximately 3 kg were sacrificed by injection of air into an ear-vein.

After opening of the abdominal wall the bladder and urethra were taken out en bloc together with the distal part of the vagina.

Morphology

Preparations obtained from the proximal two thirds of the female rabbit urethra were fixed in glutaraldehyde and processed for light and electron microscopy. Serial transverse sections were taken from each preparation. In a few preparations the urethra was not separated from the vaginal wall and transverse sections from both organs were thus obtained. A series of preparations (n=6) were processed for light microscopy after previous functional characterization and removal of the longitudinal, outer, muscle layer. Preparations for electron microscopy were after fixation in glutaraldehyde postfixed in OsO₄, embedded in Araldite, cut with glass knife and examined in a Zeiss electron microscope ($\times 2,000$). No morphological investigations were performed on human preparations.

Preparations and mounting

After removal of fat and connective tissue by combined sharp and blunt dissection two ringformed segments, each 4 mm. long, were prepared from the

proximal two thirds of the urethra. The two preparations thus obtained were suspended in 10 ml. organ baths containing Krebs solution maintained at 37°C and bubbled with a mixture of 5 per cent CO₂ and 95 per cent O₂ to give a pH of 7.4. The preparations were suspended between two L-formed hooks, one serving as an electrode and the other connected to a Grass Instruments FTO3C force transducer. The other electrode was placed immediately outside the urethral wall. The tension of the preparation could be adjusted by changing the distance between the two hooks. During an equilibration period of approximately 1 h. the tension was adjusted to a final level of 5–8 mN. The isometric tension of the preparations was recorded using a Grass Model 7D polygraph. The contractant capacity of each preparation was tested by exposure to high K⁺ solution (for composition, see below).

Electrical stimulation

Electrical stimulation was performed by means of a Grass S88 stimulation unit delivering single square wave pulses of 0.8 ms duration at a frequency of 1–50 Hz. The voltage was supramaximum and the duration of the stimulation trains was 5 sec., unless otherwise stated. The polarity of the electrodes was changed after each pulse by means of a polarity changing unit.

The frequency producing a contraction amounting to 70–80 per cent of the maximum response was chosen for the investigation. The mean of three consecutive reproducible responses (with variation <10 per cent) was regarded as representative. When quantifying the different components of the response to electrical stimulation, the maximum amplitude was measured.

6-OHDA treatment

Nine rabbits were chemically sympathectomized by means of i.v. injection of 6-hydroxydopamine-bromide. To avoid oxidation, the drug was dissolved in saline solution on ice during continuous bubbling with nitrogen. A dose of 70 mg/kg was then immediately given and the animals were sacrificed for investigation after 24 hours.

Man

The human urethral preparations were obtained from 20 male and 4 female patients with a mean age of 59 years (range 40–74 years), undergoing cystourethrectomy because of bladder malignancy. In 20/24 patients preoperative radiological treatment against the bladder (20–40 Gy) had been given.

The male urethral preparations were taken from the infracollicular, prostatic part and from the membranous part of the urethra (n=46). The other preparations from the male urethra were taken from the supracollicular prostatic part, beneath the preprostatic urethra (n=8). The preparations from the female urethra (n=11) were taken from the middle third of the organ. All preparations were taken beneath the mucosal-submucosal layers after these had been removed by sharp dissection. The preparations were taken as circularly orientated strips and were mounted in organ baths (5 or 10 ml) between two parallel stimulation electrodes, one on each side of the preparation and close to it. When rabbit preparations were investigated using this electrode location the responses were identical to those obtained with the previously described arrangement. Electrical stimulation and recording of isometric tension was performed in the same way as described for the rabbit urethral preparations. The effects of exogenously applied α -adrenoceptor stimulating

and blocking drugs were investigated in a total of 22 human preparations from twelve patients.

Solutions

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.6, CaCl_2 1.5, MgSO_4 1.15, NaHCO_3 24.9, KHPO_4 1.4, glucose 5.5. The high K^+ solution contained 124 mM K^+ and Na^+ was reduced correspondingly. In the Ca^{++} -free solution CaCl_2 was omitted and EGTA 1 mM added. All solutions were prepared the day of the experiment. Double distilled water was used and all chemicals were of analytical grade.

Drugs

Noradrenaline hydrochloride, phenylephrine hydrochloride (Sigma), clonidine hydrochloride (Boehringer-Ingelheim), phentolamine methansulphonate (CIBA-Geigy), prazosin hydrochloride (Pfizer), rauwolscline hydrochloride (Carl Roth), propranolol hydrochloride, acetylcholine chloride, atropine sulphate, scopolamine hydrochloride (Sigma), indomethacin (Dumex), 6-hydroxydopamine-bromide, tetrodotoxin (Sigma).

All concentrations given are final bath concentrations.

Results

Rabbit

Morphology

At the microscopic investigation an outer longitudinal muscle layer surrounding the anterior two thirds of the urethra was seen. In the preparations where the urethra had not been separated from the vaginal wall this layer was seen to be continuous with the vaginal muscle and surrounded this organ in a way similar to that seen in the urethra. The structure inside of the longitudinal muscle layer was a circular muscle layer, which was thicker than the former and encircled the whole organ. At the luminal side a thick submucosal layer was found. This tissue contained elastic fibers but also blood vessels, often big veins. An abundant occurrence of smooth muscle cells was also found. The muscle cells tended to form bundles deep in the submucosal layer close to the circular muscle layer. The bundles seemed to be orientated in different directions but with a longitudinal predominance. The submucosal layer formed together with the mucosa longitudinal folds. The most prominent of these was regularly found in the twelve o'clock position, the "urethral crest".

By electron microscopy it was confirmed that the longitudinal outer muscle layer consisted of smooth and striated muscle cells, while the muscle cells of the circular layer and submucosa were smooth.

Electrical stimulation

Electrical field stimulation of the rabbit urethral preparations induced a reproducible and frequency dependent contraction (Fig. 1). Threshold was at 5–6 Hz and maximum at 40–50 Hz. The maximum contractile response amounted to 43 ± 3 mN ($n=40$), comprising 93 per cent of the contraction induced by a maximum concentration of noradrenaline (NA). All electrically induced

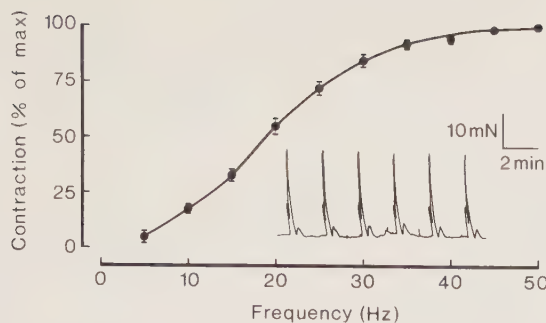


Fig 1. The frequency-response relations for the electrically induced contractant response of isolated rabbit urethral ring preparations ($n=30$) and the electrically induced contraction of an isolated rabbit urethral preparation maintained at 37°C . Stimulation frequency 30 Hz.

responses were abolished by tetrodotoxin (TTX) 10^{-6}M .

When using an increased speed of recording, it was observed that the electrically induced contraction usually consisted of several components. Typically, an initial rapidly developing phase was followed by another contraction complex, split into two peaks, after which a slower contraction component of lower amplitude was seen (Fig. 2a). However, the appearance of the contractant response varied, and in some preparations only the first component was observed (Fig. 2b). In some preparations, on the other hand, this response was absent. As can be seen in Fig. 2a, a part of the contraction developed during the stimulation period, and another part after stimulation was stopped. In preparations ($n=6$) showing both the fast and slow contraction components, removal of the outer longitudinal muscle layer by means of sharp dissection under a dissection microscope ($\times 6$) did not change the appearance of the contraction complex. That the longitudinal muscle layer had been removed was controlled microscopically.

When the stimulation experiments were performed at room temperature (20°C) ($n=4$), the initial part of the contraction could be clearly separated from the other (Fig. 2c). Occasionally this was also seen at 37°C . The relaxation within the two-peaked contraction complex (Fig. 2a) was seen immediately on stopping stimulation, and it became more pronounced when the tension of the preparation was increased, e.g. by means of NA or clonidine. Particularly when using clonidine, the relaxation was pronounced and reached maximum at a lower frequency of stimulation than when contraction had been induced by NA.

Initial components of contraction

The fast, initial contraction component reached its maximum approximately 15 times faster than the second one, and had an amplitude of 40 ± 5 per cent ($n=24$) of the maximum tension developed. It was resistant to phentolamine 10^{-6}M , and atropine 10^{-6}M , whereas the other components of contraction were reduced in a concentration dependent way, and eventually abolished by phentolamine 10^{-6}M (Fig. 3). Also after chemical sympathectomy of rabbits with 6-OHDA, the fast response was still present, whereas the other components of the contraction disappeared (Fig. 4).

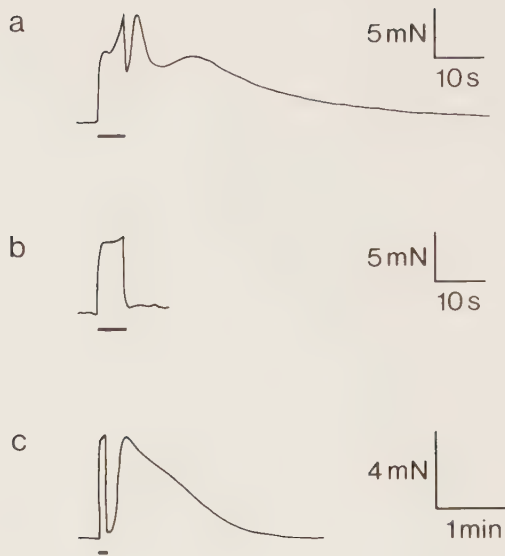


Fig 2a. High speed recording of an electrically induced contractant response of an isolated rabbit urethral preparation. Bar indicates the stimulation-train = 5 s. Note the different contractant components during and after the stimulation. Frequency 30 Hz.

Fig 2b. High speed recording of a fast contraction response followed by a rapid relaxation in an isolated rabbit urethral preparation. Bar indicates the stimulation-train = 5 s. Frequency 30 Hz.

Fig 2c. Electrically induced contraction of an isolated rabbit urethral preparation maintained at room temperature (approximately 20°C). Note that the fast and slow contractant components can be separated. Stimulation frequency 25 Hz. For comparison see Fig 1.

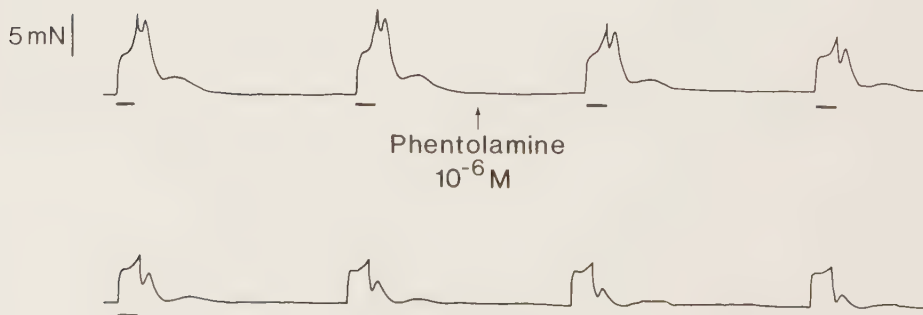


Fig 3. The effect of phentolamine $10^{-6}M$ on the electrically induced contraction of an isolated rabbit urethral preparation. Bars indicate stimulation-train = 5 s. Frequency 25 Hz.

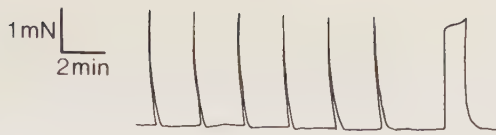


Fig 4. Typical contractant responses to electrical field stimulation of an isolated urethral preparation from a rabbit treated with 6-OHDA. The last complex was recorded at an increased paper speed. Stimulation frequency 30 Hz.

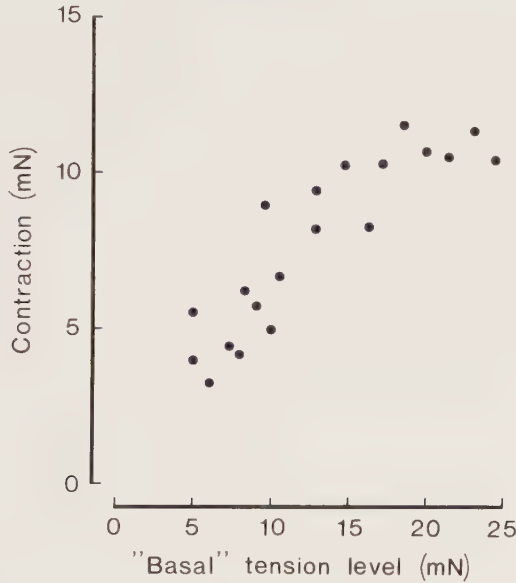


Fig 5a. The influence of noradrenaline-induced tension increase on the fast, initial contractant component of the response to electrical field stimulation in rabbit urethral preparations ($n=5$). Frequency 30 Hz.

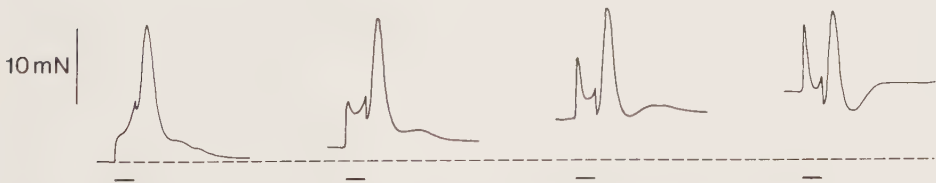


Fig 5b. The recording from one of the rabbit urethral preparations in 5a. Bars indicate stimulation-train = 5 s.

Characteristic for the initial contraction component was its dependence on the tension of the preparation, irrespective of whether this was changed passively or actively by use of, e.g., NA. The relation between the tension level and the amplitude of the fast component is illustrated in Fig. 5a. Fig. 5b shows the electrically induced contraction at different levels of tension.

At continuous electrical stimulation of preparations ($n=8$) exposed to phentolamine 10^{-6}M , or preparations from rabbits chemically sympathectomized with 6-OHDA, the contraction could be maintained. When the stimulation ceased, or when TTX 10^{-6}M was added, the preparations regained the tension level before the onset of electrical stimulation.

Late components of contraction

Except for the fast initial part, all other components of the electrically induced contraction were abolished by the non-selective α -adrenoceptor blocker phentolamine 10^{-6}M . Also the α_1 -adrenoceptor selective antagonist prazosin blocked these components in a concentration dependent way (Fig. 6), and after prazosin 10^{-6}M , only the fast initial contractant response remained. The α_2 -adrenoceptor selective antagonist, rauwolscine, 10^{-6}M , on the other hand, reduced the prazosin and phentolamine sensitive part of the contraction by only 26 ± 3 per cent ($n=15$) (Fig. 6). Also atropine reduced the contractant components sensitive to prazosin and phentolamine in a concentration-dependent way. The maximum inhibitory effect, 53 ± 3 per cent ($n=10$), was obtained at a concentration of 10^{-5}M .

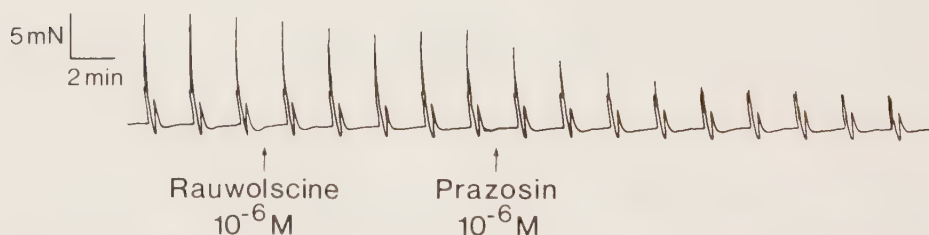


Fig 6. The effects of the α_2 - and α_1 -adrenoceptor blocking agents rauwolscine 10^{-6}M and prazosin 10^{-6}M on the electrically induced contractant response in an isolated female rabbit urethral preparation. Stimulation frequency 30 Hz.

Scopolamine was less active than atropine, producing an inhibition of 22 ± 3 per cent ($n=6$) at 10^{-5}M . In the presence of acetylcholine 10^{-5}M – $5\times 10^{-4}\text{M}$, concentrations which per se had no contractant effect on the preparations, a transient increase of the late components of contraction amounting to 10 ± 4 per cent ($n=6$) was found. Propranolol 10^{-8} – 10^{-6}M had no effect.



Fig 7. The dependence of the electrically induced fast and slow contractant components of the rabbit urethra on extracellular Ca^{++} . Ca^{++} -free Krebs solution containing EGTA 1 mM introduced at first arrow and Ca^{++} -containing Krebs solution reintroduced at the second arrow. Stimulation frequency 25 Hz. Note the different Ca^{++} -dependence of the two contraction components.

Ca²⁺ -dependence

The dependence on the presence of extracellular Ca²⁺ was found to differ between the fast, initial and the following components of contraction. The initial response was blocked by 38 ± 6 per cent ($n=4$) after exposure to Ca²⁺ - free solution for 40 min., while the other contractant components were reduced by 57 ± 4 per cent already after 10 min. ($n=6$; Fig. 7), and abolished after approximately 20 min.

Man

Electrical stimulation

Electrical field stimulation induced reproducible and frequency dependent contractant responses in preparations from both the male and female urethra (Fig. 8). The contraction complexes had similarities to that observed in the

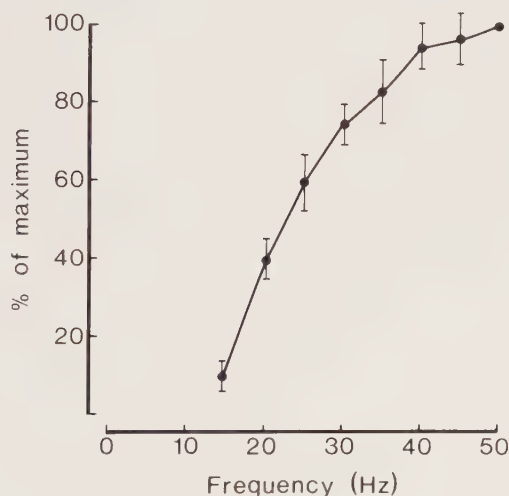


Fig 8. The frequency-response relations for the electrically induced contractant response of isolated human urethral preparations ($n=7$).

female rabbit urethra, and consisted of an initial contraction followed by relaxation (sometimes below the initial tension level, Fig. 9a). In another few preparations, from both the male and female urethra, the fast initial contraction was immediately followed by an equally fast relaxation to base-line, when stimulation was stopped (Fig. 9b). This response was resistant to scopolamine 10^{-5} M and phentolamine 10^{-6} M, but abolished by TTX 10^{-6} M (Fig. 10). In preparations from the prostatic urethra no relaxation below base-line was observed. As in rabbit, the late contraction components of the human urethral preparations were sensitive to α -adrenoceptor blockade. A reduction by 86 ± 7 per cent ($n=7$) was seen after phentolamine 10^{-6} M or prazosin 10^{-6} M. In the presence of clonidine a reduction of the contractant response to electrical field stimulation by 83 ± 8 per cent at 10^{-6} M ($n=5$) was seen in preparations from both the prostatic and membraneous parts of the urethra (Fig. 11).

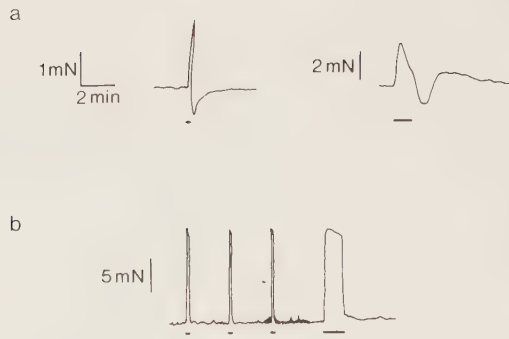


Fig 9a+b. Low and high speed recordings of the electrically induced contractant responses of isolated human urethral preparations from the infracollicular part of the urethra. Frequency 20–30 Hz. Bars indicate stimulation-train = 5 s.

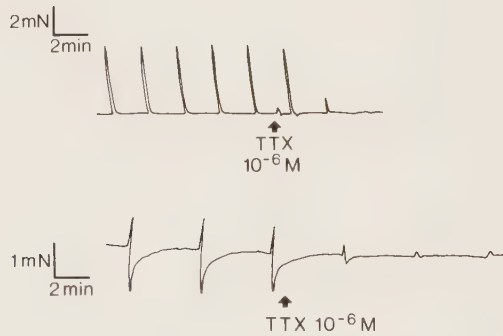


Fig 10. Electrically induced responses of isolated human female (upper curve) and male (lower curve) urethral preparations, taken from the zones representing the maximum urethral pressure. The preparations were exposed to prazosin 10^{-6} M rauwolscine 10^{-6} M and atropine 10^{-5} M (female preparation) phentolamine 10^{-6} M and scopolamine 10^{-5} M (male preparation) with contact times of 15 minutes at the time of recording. TTX = tetrodotoxin. Stimulation frequency 15 Hz.

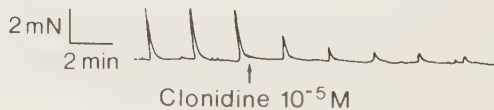


Fig 11. The effect of the α_2 -adrenoceptor stimulating agent clonidine 10^{-5} M on the electrically induced contractant response of an isolated human male urethral preparation from the infracollicular part of the urethra. Stimulation frequency 20 Hz.

Also atropine induced a concentration dependent reduction of the electrically induced contraction by maximum 58 ± 10 per cent ($n=6$) at a concentration of 10^{-5} M. Scopolamine in a concentration of 10^{-5} M reduced the phentolamine sensitive electrically induced response by 39 ± 6 per cent in six preparations from the infracollicular part of the urethra (Fig. 12), while no effect was seen in

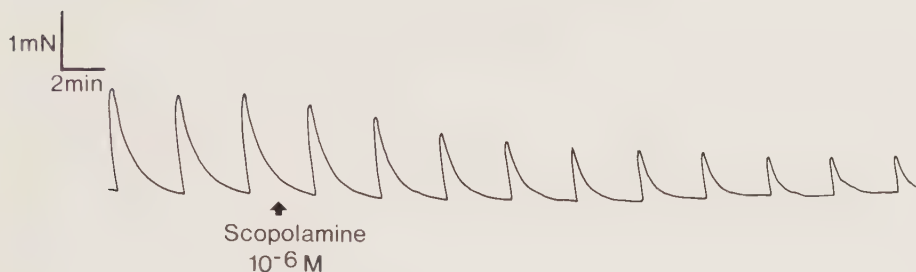


Fig 12. The effect of scopolamine 10^{-6} M on the electrically induced contractant response of an isolated human male urethral preparation from the infracollicular-membraneous part of the urethra. Stimulation frequency 25 Hz.

another three preparations. In preparations with a fast initial contractant response this could not be blocked by phentolamine 10^{-6} M or atropine 10^{-5} M. Attempts to reduce or abolish the response to electrical stimulation of human preparations ($n=18$) by TTX revealed a considerable variation in sensitivity to this agent. Relaxant responses were always abolished, whereas contractant responses were abolished in 12 preparations and in 2 reduced by approximately 60 per cent. In 4 preparations even an increase of the contractant response was seen.

Direct effects

Clonidine 10^{-8} – 10^{-6} M per se had a direct contractant effect in preparations from only one out of eight patients, amounting to 8 ± 2 per cent ($n=3$) of the contraction induced by a maximum concentration of NA. In 16 preparations from 7 other patients, clonidine was without effect, while NA and the α -adrenoceptor stimulating agent phenylephrine readily contracted the preparations. The NA-induced contractant responses were blocked by 84 ± 5 per cent ($n=5$) in the presence of prazosin 10^{-6} M, while rauwolscine 10^{-6} M was without effect ($n=5$).

It should be noted that acetylcholine 10^{-5} M induced direct contractant effects on preparations taken from the prostatic part of the urethra, amounting to 14 ± 5 per cent ($n=5$) of the maximum NA-induced contraction; in two other preparations, however, the contraction was 75 and 50 per cent. Preparations from the infracollicular part of the male urethra ($n=10$) only occasionally showed a small (<10 per cent) contractant response to acetylcholine.

Discussion

The present investigation showed that the electrically induced contraction of the urethral muscle from rabbit and man consisted of two main components, one adrenergically mediated, and one non-adrenergic, atropine resistant. A

similar, two-component contractant response has been demonstrated in, e.g., the rat vas deferens¹²⁻¹⁴. It is known that in both sexes, the human urethra contains within its walls the external urethral sphincter consisting of striated muscle fibres¹⁵. In the present study, the rabbit urethral smooth muscle investigated contained both smooth and striated components. Striated muscle cells were, however, found only in the longitudinal muscle layer. The appearance of the electrically induced contraction-complex was not dependent on the longitudinal muscle layer and, thus, could not be attributed to the striated muscle component.

The adrenergic response

McGrath¹⁴ found that the mechanical response of the longitudinal muscle of rat vas deferens to stimulation of its motor nerves by a single pulse, consisted of two components, one developing faster than the other. The second of these components of contraction, but not the first, was abolished by α -adrenoceptor blockade, potentiated by drugs inhibiting neuronal uptake of NA, and absent in tissue pre-treated with reserpine. It was therefore attributed to released NA. In the present study a considerable part of the electrically evoked urethral contraction in preparations from both rabbit and man could be blocked by phentolamine and, as shown in the rabbit, also eliminated by treatment with 6-OHDA. These findings favour the view that also in the rabbit and human urethra this contraction component is attributable to NA and that NA released from adrenergic nerve endings comprise an important mechanism for activation of the urethral smooth muscle. This is in line with findings in previous investigations¹⁶. Although the α -type of α -adrenoceptor seems to dominate quantitatively in the rabbit urethra and also to mediate contraction¹⁷, the α_1 -adrenoceptor antagonist prazosin was able to inhibit the electrically induced contraction more effectively than the α_2 -adrenoceptor antagonist rauwolscine. This discrepancy may indicate different localizations and/or functions of the two types of postjunctional α -adrenoceptor in the rabbit urethra. As suggested for vascular smooth muscle¹⁸, it might be that in the rabbit urethra α_1 -adrenoceptors are located intrajunctionally and are important for mediation of the nerve-evoked contraction, and the α_2 -adrenoceptors are situated outside the neuromuscular junction and influenced primarily by circulating catecholamines.

Also in the human urethra, the electrically induced contraction could be blocked effectively with phentolamine. Unlike the situation in the rabbit female urethra¹⁷ clonidine had little direct contractant effect on the human urethral preparations and rauwolscine did not inhibit NA-induced contractant responses. However, clonidine effectively inhibited the electrically induced contraction of human urethral smooth muscle, an effect probably mediated via stimulation of α_2 -adrenoceptors on adrenergic nerve endings with consequent inhibition of NA-release. Stimulation and blockade of prejunctional α_2 -adrenoceptors have been shown to influence the electrically induced release of ³H-NA from isolated urethral smooth muscle from both rabbit and man¹⁹.

In the human urethra, the proportions of α_1 - and α_2 -adrenoceptors have not yet been established. However, judged by the direct effects of clonidine, and rauwolscine postjunctional contraction-mediating α_2 -adrenoceptors seem to have no important function.

Also drugs exerting their effects on muscarinic cholinceptors were found to significantly affect the adrenergic component of the electrically induced contraction, acetylcholine increasing and atropine and scopolamine decreasing this

contraction component. The effects of atropine in the rabbit urethra in concentrations $\geq 10^{-6}$ M have been shown to be exerted partly via an α -adrenoceptor blocking effect while this was not the case for scopolamine²⁰. The pronounced effects of scopolamine suggest that muscarinic receptor mediated activity can be of importance for the activation of urethral smooth muscle. However, the present information on urethral effects of cholinergic active drugs seems difficult to interpret. The release of 3 H-NA from adrenergic nerve endings was significantly decreased by acetylcholine and increased by atropine¹⁹. This would favour the view that the effects of these drugs on the electrically induced contraction are exerted postjunctionally. On the other hand, the direct effects of acetylcholine on rabbit and human urethral muscle were small. Even if these *in vitro* data cannot be extrapolated to the *in vivo* situation, it should be brought in mind that investigations in man have shown neither cholinergic agonists nor antagonists to significantly influence intraurethral pressure at rest^{4,11}.

The non-adrenergic atropine-resistant response

The non-adrenergic character of the initial rapidly developing contraction was shown by the fact that it was influenced neither by α -adrenoceptor blockade nor by chemical sympathectomy with 6-OHDA. Furthermore, the experiments performed at room temperature showed that it was quite distinct from the α -adrenoceptor mediated response. The response could be completely blocked by tetrodotoxin and was thus most probably mediated via nerves. The origin and the nature of the putative transmitter is not known, but the experiments with preparations from 6-OHDA treated animals seem to rule out adrenergic nerves as the source. A striking characteristic was that the response developed rapidly during the stimulation period and that the preparations relaxed almost immediately to base line on cessation of stimulation. The dependence of the amplitude of this contraction on the basal tension level and also on the NA-induced increased tension level was interesting to note. It is tempting to speculate that this mechanism might be of importance for the fast, nerve-mediated reflex response in the urethra seen after sudden rise of the intraabdominal pressure^{21,22}. The response was quite resistant to deprivation of extracellular calcium compared to that mediated via α -adrenoceptors.

In some preparations only a non-adrenergic type of contraction could be elicited, and in some a response that could be completely blocked by α -adrenoceptor agonists was found. Both extremes were more common in human than in rabbit preparations. This may be explained by species differences, but also by the possibility that the human material was affected by factors such as irradiation therapy and surgical trauma. It cannot be excluded that the fact that in the rabbit urethra all layers of the wall were represented while the human preparations comprised only a part of the urethral wall also may be of importance. It is important to note, however, that the same types of contractile activation mechanisms could be identified in rabbit and human urethral smooth muscle.

The present results thus suggest that the electrically induced adrenergic activation of urethral smooth muscle in both rabbit and man is mediated mainly via α_1 -adrenoceptors. A non-adrenergic atropine-resistant contractant response mediated via an unknown transmitter was also found in the urethra of both rabbit and man. Whether this response is an expression for activity mediated via nerves in the parasympathetic, sacral pelvic nerves or if it is an expression for the presence of another type of nerves remains to be established.

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II

ELECTRICALLY INDUCED RELAXATION OF THE NORADRENALINE CONTRACTED ISOLATED URETHRA FROM RABBIT AND MAN

K.-E. ANDERSSON, A. MATTIASSON AND C. SJÖGREN*

From the Departments of Clinical Pharmacology and Urology, University Hospital, Lund Sweden, and Research Laboratories, AB Leo, Helsingborg, Sweden

ABSTRACT

Isolated urethral preparations from rabbit and man responded to transmural electrical stimulation with contraction when the basal tension was low, and with relaxation when the preparations were contracted by noradrenaline, clonidine, 5-hydroxytryptamine and lysine vasopressin. Both contractant and relaxant responses were abolished by tetrodotoxin, suggesting that they were caused by the action of transmitters released from nerves. The electrically induced contractions in the rabbit urethra had a threshold frequency of 5 to 6 Hz and maximum was reached at 40 Hz. The responses were markedly reduced by α -receptor blockers suggesting that the released contraction-mediating transmitter was mainly noradrenaline. The relaxant response was immediate and rapidly reversible. It was obtained at a threshold frequency of 1 to 2 Hz and maximum was reached at 10 to 15 Hz. It was not inhibited by propranolol, indomethacin, atropine or peptides such as substance P, VIP, vasopressin or somatostatin. Prostaglandin E_2 , isoprenaline, adenosine-5'-triphosphate and VIP relaxed the noradrenaline contracted rabbit urethra with a time course different from that of the electrically induced relaxation. Nifedipine and "calcium free" solution decreased the noradrenaline induced contraction but did not influence the relaxant response to electrical stimulation. It is suggested that the relaxant response of the isolated noradrenaline-contracted urethra to electrical stimulation is caused by an unknown transmitter released from nerves.

MATERIALS AND METHODS

Tanagho and Miller¹ described the normal pattern of initiation of voiding in man as an initial drop in urethral pressure followed 5 to 15 seconds later by detrusor contraction. Their findings were confirmed in later investigations.²⁻⁵ The mechanism behind the fall in urethral pressure is not known, but may be attributed to several factors. Sympathetically controlled smooth muscle tone has been shown to be an important factor in the maintenance of intraurethral pressure,^{6,7} and a decrease in sympathetic nervous discharge may therefore reduce this pressure. The distribution of adrenoceptors in the urethra with a predominance of contraction-mediating α -receptors⁸ also makes a reduction in α -receptor mediated tone an attractive explanation to urethral relaxation. Based on sacral root stimulation experiments in cats, McGuire and Herlihy⁹ considered such an explanation unlikely, and suggested a decrease in urethral closure pressure to be a response to parasympathetic discharge, which ultimately involved β -receptor activation.⁴ However, there is no known transmitter with effects on urethral β -receptors only. Thus, released noradrenaline could be expected to produce contraction of the urethra. On the other hand, it cannot be excluded that there is a release from urethral nerve endings of a transmitter with relaxant effects on urethral smooth muscle. The prostaglandins E_1 and E_2 cause relaxation of the human urethra both *in vitro*^{10,11} and *in vivo*,¹² but whether or not they are released or have a role in normal micturition is not known.

In order to investigate the possibility that a relaxation mediating transmitter is released from nerve terminals in the urethra, the present study was performed. Isolated urethral preparations from rabbit and man were electrically stimulated when the resting tension was low and when the preparations were contracted by noradrenaline, clonidine, 5-hydroxytryptamine and lysine vasopressin.

Rabbit. Sixty female rabbits with an average weight of 3 kg. were stunned by a blow on the back of the head and exsanguinated. The bladder and urethra were immediately dissected free down to the entrance of the vaginal wall and removed. The bladder was opened and the trigonal area and the internal urethral orifice were identified. Two circular transverse sections, each 4 mm. long, were taken from the middle and upper parts of the urethra. The ring-formed preparations were transferred to a 30 ml. temperature controlled organ bath filled with Krebs's solution (composition, see below) that was maintained at 37°C and bubbled with a mixture of 95 per cent O_2 and 5 per cent CO_2 to give a pH of 7.4. The preparations were mounted on 2 L-shaped platinum hooks placed within the lumen of the urethral ring (fig. 1). The upper hook was connected to a force transducer (Statham FT 03) for registration of isometric tension. The lower hook was fixed to a movable unit allowing adjustments of tension; this hook was also used as an electrode for electrical stimulation. The other platinum electrode was S-shaped and placed close to the preparation. The urethral rings were repeatedly stretched and were then allowed to relax until a stable tension level was obtained. After an equilibration period of 60 minutes, tension was adjusted to approximately 8 mN.

Man. The human urethral preparations were obtained from 7 male patients undergoing cystourethrectomy en bloc because of bladder cancer. Radiological treatment in a dose of 20 Grey was given to these patients the week before operation. Rings of tissue were taken from the membranous and supra- and infracolicular parts of the prostatic urethra. The rings were opened, the mucosa removed, and the underlying tissue carefully dissected into strip preparations approximately 1 to 2 mm. wide, 0.5 to 1 mm. thick and 8 to 10 mm. long. All preparations were mounted in thermostatically controlled organ baths filled with Krebs's solution (composition, see below) kept at 37°C and bubbled with a mixture of 95 per cent O_2 and 5 per cent CO_2 . The preparations were allowed an equilibration period of 1 to 1.5

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* Requests for reprints: Research Laboratories, AB Leo, S-251 09 Helsingborg, Sweden.

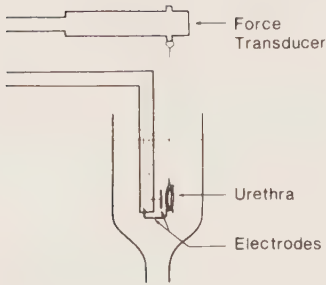


Fig. 1. Schematic drawing of the organ bath for transmural electrical stimulation of rabbit urethral rings.

hours. They were repeatedly stretched until a stable tensior level of approximately 8 mN was obtained. Isometric tension was recorded by means of Satham FT03 force transducers connected to a Grass model 7 polygraph.

Electrical stimulation. The current used for electrical stimulation was delivered by a Grass stimulator, model S44. The preparations were stimulated by single square wave pulses of 0.8 msec. duration and a frequency of 1 to 40 Hz. The voltage was supramaximum (voltage over the electrodes approx. 4 V), train duration was 5 seconds, and the stimulation interval 2 minutes. All excitatory responses at a low basal tension were induced by frequencies evoking a contraction 70 to 80 per cent of maximum. In some experiments the stimulation was also performed continuously at 2 to 10 Hz. The urethral preparations were electrically stimulated before and after they were contracted by noradrenaline, clonidine, 5-hydroxytryptamine or lysine vasopressin. The contraction capacity of each preparation was initially tested by exposure to noradrenaline (8 and 60 μ M).

Solutions. The Kreb's solution used had the following composition (mM): NaCl 118, KCl 4.6, CaCl_2 1.5, MgSO_4 1.15, NaHCO_3 24.9, KH_2PO_4 1.4 and glucose 5.5.

In some experiments a high potassium solution was used. The composition was the same as in normal Kreb's solution, except that it contained 127 mM K^+ and Na^+ was reduced correspondingly.

Drugs. Noradrenaline hydrochloride, isoprenaline hydrochloride, propranolol hydrochloride, acetylcholine chloride, histamine dihydrochloride, 5-hydroxytryptamine creatinin sulphate complex, atropine sulphate, adenosine-5'-triphosphate (ATP), adenosine, prostaglandin E_2 (PGE_2), tetrodotoxin, substance P, somatostatin (Sigma); neurotensin, vasoactive intestinal peptide (VIP) (Cambridge Research Biochemicals), clonidine hydrochloride (Boehringer-Ingelheim); nifedipine (Bayer AG); metoclopramide hydrochloride (Lundbeck A/S), indomethacin (AB Leo), prazosin hydrochloride (Pfizer AB), 8-lysine vasopressin, angiotensin (Sandoz AG), and phentolamine methanesulphonate (CIBA-Geigy).

RESULTS

Rabbit. At the normal ("low") basal tension (~8 mN), the preparations responded to electrical stimulation with a contraction. The response was frequency-dependent with a threshold frequency of 5-6 Hz (figs. 2 and 3). The maximum tension developed was obtained at 40 Hz and generally had the same amplitude as the contraction induced by a maximum concentration of noradrenaline (39 ± 4 mN and 41 ± 4 mN, respectively, mean $\pm$ standard error, $n = 40$). The electrically induced contractions were abolished by tetrodotoxin 1 μ M. Non-selective α -receptor blockade by phentolamine 1 μ M or by a combination

of prazosin 1 μ M and rauwolfscine 1 μ M inhibited the responses by a mean of 72 ± 6 ($n = 22$). Also atropine at a concentration of 1 μ M (exposure time 10 to 15 minutes) had an distinct inhibitory action both in untreated preparations and in those pretreated with α -receptor antagonists, depressing the contractions by 20 to 50 per cent ($n = 10$). However, acetylcholine per se in concentrations up to 10 μ M had no or a very slight contractant effect ($n = 120$). Noradrenaline 60 μ M, added to the organ bath, produced contractions that were maintained at a stable level for at least 10 to 15 minutes.

Electrical stimulation of noradrenaline contracted preparations induced a pronounced relaxation (fig. 2). This relaxant response was often, but not always, preceded by a fast and transient contraction. The relaxation was frequency-dependent, the threshold frequency being 1 to 2 Hz. Maximum relaxation was 57 ± 2 per cent ($n = 30$), of the tension produced by noradrenaline, and reached at 10 to 15 Hz (fig. 3). The prestimulation level of tension was reached within 1 minute after cessation of stimulation. The relaxant response was generally greater in preparations from the distal than from the proximal part of the urethra. Tetrodotoxin 1 μ M abolished the relaxant responses, but lacked effect on the contraction induced by noradrenaline 60 μ M.

High potassium solution (127 mM K^+) evoked a strong and sustained contraction of the preparations ($n = 5$). Electrical

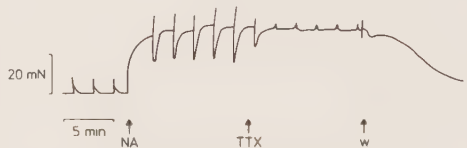


Fig. 2. Electically induced contractant response of rabbit urethra becomes evident when the basal tension of the preparation is low (~7 mN). The relaxant response appears when the preparation is contracted by noradrenaline (NA, 60 μ M). Tetrodotoxin (1 μ M, TTX) abolishes the electrically induced relaxation. Supramaximum voltage, 20 Hz, 0.8 msec. duration. w = wash.

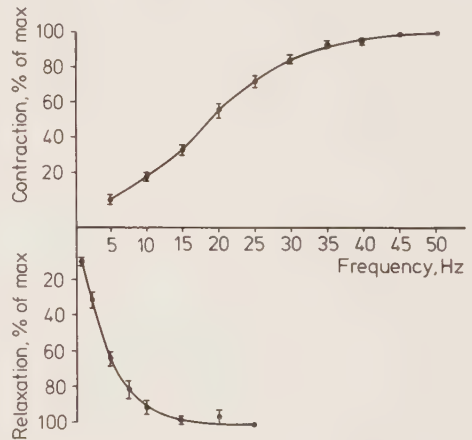


Fig. 3. Frequency-response curves for the contractant and relaxant responses of the electrically stimulated rabbit urethra. Each point on the curves represents the mean $\pm$ standard error of 30 to 45 values.

stimulation caused no or a very slight contraction; there was no relaxant effect (fig. 4).

When noradrenaline was added cumulatively to the urethral rings, the contractant effect of electrical stimulation seen at a low basal tension first tended to increase and was then successively reduced. The relaxant response was observed when the tension exceeded approximately 12 mN (corresponding to about 8 μ M noradrenaline) and increased successively with increasing tension levels (fig. 5).

By continuous electrical stimulation (2 to 10 Hz) of preparations contracted by noradrenaline, it was possible to maintain the relaxant response at a constant level for at least 10 minutes. When the stimulation was interrupted or when tetrodotoxin 1 μ M was added (n = 4), the tension of the preparation returned to the starting level (fig. 6).

The relaxant response to electrical stimulation was not influenced by pretreatment with propranolol 1 μ M (n = 4), atropine 1 μ M (n = 4), metoclopramide 10 μ M (n = 4), indomethacin 18 μ M (n = 4), substance P 0.1 μ M (n = 5), VIP 0.5 μ M (n = 4), angiotensin 0.1 μ M (n = 4), lysine vasopressin 10^{-4} IE/ml. (n = 4) or somatostatin 1 μ M (n = 4). In the noradrenaline contracted urethra, isoprenaline 0.1 μ M (n = 5), adenosine 1 mM (n = 3), ATP 1 mM (n = 4), PGE₂ 5.7 μ M (n = 3) and VIP 0.5 μ M (n = 5) produced slowly developing and incomplete (20 to 40 per cent) relaxations. Prazosin 1 μ M (n = 5) also had a slowly developing relaxant effect. Lysine vasopressin 1 IE/ml. (n = 4) caused a further increase in tension. The following substances had neither contractant nor relaxant effects on urethral rings contracted by noradrenaline: acetylcholine 1 μ M (n = 10), his-

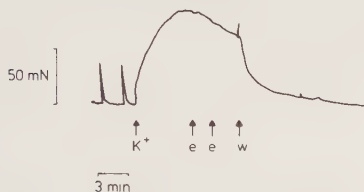


FIG. 4. The contractant response of rabbit urethra to electrical stimulation at low basal tension (8 mN) is seen initially. After addition of potassium (127 mM, K⁺) electrical stimulation (e) is without effect. Supramax. voltage, 15 Hz, 0.8 msec. duration. w = wash.

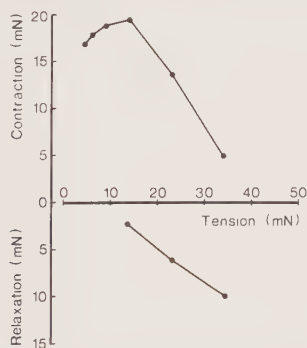


FIG. 5. Amplitudes of the contractant and relaxant responses to electrical stimulation in an isolated rabbit urethra. The responses were recorded at a low resting tension (~5 mN) and when the preparation was contracted by cumulative addition of noradrenaline (1, 2, 4, 8, 30 and 60 μ M) producing a successive increase in tension. Supramax. voltage, 30 Hz, 0.8 msec. duration.

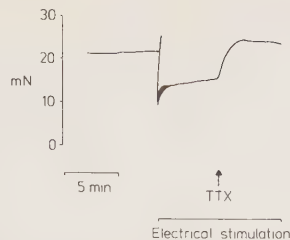


FIG. 6. Recording from an isolated rabbit urethra contracted by noradrenaline (60 μ M). A sustained relaxation occurs at continuous electrical stimulation (supramax. voltage, 8 Hz, 0.8 msec. duration). Addition of tetrodotoxin (TTX 1 μ M) reestablishes the tension to the prestimulation level.

tamine 0.1 to 1.0 μ M (n = 4), metoclopramide 0.1 to 1.0 μ M (n = 4), somatostatin 1 μ M (n = 4), substance P 0.1 μ M (n = 5), angiotensin 0.1 μ M (n = 4) and neurotensin 0.5 μ M (n = 2).

Like noradrenaline, clonidine (16 μ M; n = 8), lysine vasopressin (10^{-3} IE/ml; n = 5) and 5-hydroxytryptamine (10 μ M; n = 4) contracted the preparations. Electrical stimulation evoked a reproducible relaxant response in all preparations tested with the same appearance as described above. The relaxant responses were uninfluenced in preparations pretreated with phentolamine (1 μ M; n = 3) and then contracted by lysine vasopressin or 5-hydroxytryptamine, but was blocked by TTX (1 μ M). The relaxant response to electrical stimulation was also seen in preparations in which the tension was raised by clonidine.

Omitting calcium from the bath solution conspicuously reduced the noradrenaline-induced contraction (n = 3). The relaxant response to electrical stimulation was also reduced expressed in absolute values. However, it was not reduced if related to the reduction of the noradrenaline-induced contraction.

The calcium entry blocker nifedipine 1 μ M (n = 4) did not influence the relaxant response to electrical stimulation but slightly reduced the noradrenaline-induced contraction.

Man. At "low" basal tension (~8 mN), preparations taken from the supracollicular part of the prostatic urethra responded with contraction to electrical stimulation. The response was frequency-dependent and reached a maximum at 40 Hz. In all preparations (n = 12) from the infracollicular part of the urethra, contracted by noradrenaline 60 μ M, electrical stimulation caused a relaxation similar to that observed in the rabbit urethra (fig. 7). However, this relaxant response was not seen in noradrenaline contracted preparations from the supracollicular part of the organ (n = 9), which responded with a further contraction. At a low basal tension, the infracollicular part of the urethra responded to electrical stimulation with relaxation in preparations from 2 out of 7 patients and with contraction in the other 5 patients.

DISCUSSION

The present results showed that in isolated smooth muscle from the urethra of rabbit and man, transmurial electrical stimulation caused a contraction when the resting tension of the preparations was low. This response was abolished by tetrodotoxin, suggesting neuronal release of a contraction-producing transmitter. The contractions were markedly but not completely reduced by α -receptor blockers indicating that the released transmitter was mainly noradrenaline. These findings are in line with current concepts of urethral innervation and receptor functions. The smooth muscle of the urethra receives adrenergic as well as cholinergic nerves¹⁰⁻¹⁵ and there is a functional predominance of α -receptors.^{8, 11, 16, 17} In man, choli-

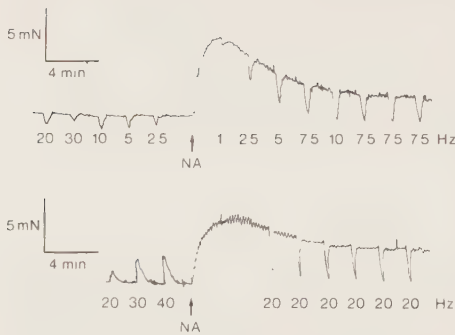


FIG. 7. The electrically induced responses at different frequencies in preparations taken from the human infracolicular, prostatic urethra at a low basal tone (9 mN) and after contraction induced by noradrenaline (NA) 60 μ M. Note the relaxant response obtained even at a low basal tone in the upper recording

noceptor stimulation and blockade have little effect on the urethra, which has been shown both *in vitro*^{18,19} and *in vivo*. As acetylcholine had no effect *per se*, the inhibitory effect of atropine seen in the rabbit urethra is difficult to explain. A presynaptic effect on muscarinic receptors located at adrenergic nerve terminals,²⁰ could rather be expected to produce an enhancement of the responses by increasing noradrenaline release.

When the preparations were contracted by noradrenaline, which may mimic a continent situation, electrical stimulation produced relaxation. This response was prevented if tetrodotoxin was present when stimulation was performed, or immediately counteracted if tetrodotoxin was added when relaxation had been induced. This suggests the involvement of nerves and the release of a relaxation producing transmitter. The experiments with phentolamine pretreatment of lysine vasopressin or 5-hydroxytryptamine contracted preparations seem to separate the relaxant response from α -receptor mediated contraction. When the preparations were contracted by potassium, no relaxation was produced, suggesting either that there was no transmitter release from the depolarized nerves, and/or that the potassium-depolarized muscle was unresponsive to the transmitter.

It has been shown that beside the adrenergic and cholinergic nerves the bladder and urethra contain fibers which differ from the classical types of autonomic nerves. These nerves may be called purinergic,^{21,22} or peptidergic.²³ Peptides, e.g., substance P²⁴ and VIP,^{25,26} have been demonstrated in the genitourinary tract in several species.

The nature of the putative transmitter in the present experiments is unclear. It is known that the prostaglandins E_1 and E_2 as well as the β -receptor agonist isoprenaline have a relaxant effect on isolated urethral muscle from both animals²⁷ and man.^{10,12} Also ATP relaxes isolated contracted guinea-pig urethra.²⁸ The time course of the relaxant effect of the prostaglandins and of isoprenaline, as found in the present investigation, was different from that induced by electrical stimulation. Furthermore, the electrically induced relaxation was not blocked by indomethacin or by propranolol, suggesting that neither prostaglandins, nor β -receptors were involved in the response. ATP and adenosine in the concentrations used (up to 1 mM) had a slowly developing and incomplete relaxant effect on noradrenaline contracted preparations. This does not favor their involvement as transmitters for the relaxant response, but does not completely exclude such an action, as the effects of substances added to the organ bath do not necessarily reflect their action when released at the receptor site.

Several other drugs known to be involved in or to affect transmitter functions, e.g., acetylcholine, atropine and metoclopramide did not, in the concentrations used, influence the relaxant response.

The frequency threshold for the electrically evoked contraction was 5 to 6 Hz, and maximum response was obtained at approximately 40 Hz. In contrast, the threshold for relaxation was 1 to 2 Hz and the maximum response was obtained at 10 to 15 Hz. It is known from experiments with other tissues that different transmitters may be released when different stimulation parameters are used.²⁹ However, neither of the substances tested, including those whose presence in the urethra has been demonstrated, i.e. substance P, VIP and somatostatin, closely mimicked or inhibited the electrically induced relaxation. VIP had a relaxant effect on the urethra, and the involvement of this peptide in the relaxant response cannot be excluded by the present experiments, but requires further studies.

The relaxant response was immediate and also rapidly reversible; within a minute of cessation of stimulation the preparations had regained their pre-stimulation tension level. Assuming that there is a neuronal release of a transmitter, this finding favors the existence of a transmitter-inactivating mechanism.

It might thus be that during transmural electrical stimulation of the urethra, both excitatory and inhibitory transmitters are released from urethral nerves. When the tension is low, the effect of the excitatory transmitters (probably mainly noradrenaline), predominates. When the tension is increased, which may mimic the physiologic situation in a continent individual, the effect of the relaxant transmitter is demonstrable.

Whether this relaxant mechanism contributes to the fall in intraurethral pressure preceding, and persisting during normal bladder emptying,^{1,3-5} or if it has any pathophysiologic significance remains to be established.

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III

Adrenoceptors and Cholinoceptors Controlling Noradrenaline Release from Adrenergic Nerves in the Urethra of Rabbit and Man

by

Anders Mattiasson, Karl-Erik Andersson and Christer Sjögren

Departments of Urology and Clinical Pharmacology, University Hospital, Lund, Sweden

Abstract

The possible influence of adreno- and cholinoceptors on noradrenaline (NA) release from adrenergic nerve endings in urethral smooth muscle of rabbit and man was investigated in preparations preincubated with ^3H -NA. The preparations were continuously perfused in a temperature controlled (37°C) chamber and the amount of electrically induced efflux of ^3H was calculated as a fraction of the total amount of ^3H in the preparation at the time of stimulation. In preparations from both rabbit and man, the fractional release of ^3H induced by electrical field stimulation was reproducible, and significantly increased in the presence of the α_2 -receptor antagonist rauwolscline, and the muscarinic cholinoreceptor antagonists scopolamine and atropine. There was a significant decrease of ^3H -release in the presence of the α_2 -receptor agonist clonidine and of the cholinoreceptor agonist carbachol. No evidence for the presence of prejunctionally located α_1 - or nicotinic receptors was found.

The results suggest the possibilities of 1/ a negative feed-back regulation of the release of noradrenaline from adrenergic nerve endings in the urethra, mediated via prejunctionally located α_2 -adrenoceptors, 2/ an interaction at the nerve terminal level between adrenergic and cholinergic nerves mediated via muscarinic cholinoceptors on the adrenergic nerve terminals.

Introduction

Urethral smooth muscle is generally considered to be of great importance for the maintenance of urinary continence (1–3). The tone of this muscle is believed to be controlled mainly through adrenergic nerves and mediated via α -adrenoceptors (1–3). Theoretically, α -adrenoceptors located both pre- and postjunctionally may be involved in this control. Using radioligand binding technique, both α_1 and α_2 -adrenoceptors have been demonstrated in the female rabbit urethra with a quantitative predominance of α_2 -adrenoceptors, probably located mainly postjunctionally (4). In man, the functional significance and the location of the two types of α -adrenoceptor have not been

established, although in vitro and in vivo studies have suggested postjunctional α_1 -adrenoceptors to be functionally important (5). In studies of several tissues with adrenergic innervation, the presence of prejunctional α - and β -adrenoceptors participating in the regulation of the release of noradrenaline from adrenergic nerve terminals have been demonstrated (see, e.g. Langer 1981) (6). One aim of the present study was therefore to investigate whether the receptor basis for such autoregulatory mechanisms could be demonstrated in urethral smooth muscle from rabbit and man.

It is well known that the urethra of both animals and man has a relatively rich supply of cholinergic nerves (7). Acetylcholine did not produce any significant contraction of human urethral muscle strips in vitro (8), neither did cholinergic stimulating agents give any increase of the intraurethral pressure in vivo (3). There is, however, strong support from both morphological and functional studies for an interaction to occur at the ganglionic level between adrenergic and cholinergic nerves supplying the lower urinary tract (7, 9, 10). On morphological basis an interaction also at the nerve terminal level has been suggested (7, 10). In the present study the possibility to influence noradrenaline release via cholinergic receptors on adrenergic nerve endings in the urethra of rabbit and man was investigated.

Material and methods

Preparations

Female rabbits (Danish land race, $n=41$) weighing approximately 3 kg were sacrificed by injection of air into an ear-vein. The abdominal cavity was opened and, after gentle dissection, the bladder and urethra were removed en bloc together with the distal part of the vagina. The bladder and urethra were opened longitudinally and the urethra was cut close to the entrance into the vaginal wall and at the level of the internal urethral orifice.

The urethral mucosa as well as periurethral fat and connective tissue were removed by sharp dissection. The urethral tissue specimens were divided longitudinally into two identical pieces, each of which weighed approximately 100 mg. Each piece of the urethral smooth muscle was cut into 8 one mm wide interconnecting strips, comprising material for two experiments, and incubated in a glass tube with ^3H -NA 1 μM (2.7 Ci/mmol, New England Nuclear) in Krebs solution bubbled with a mixture of 5% CO_2 and 95% O_2 at 37°C (pH 7.4) for 45 min. Separate experiments showed that maximum uptake of ^3H was reached within this time. A total number of 164 preparations from the rabbit female urethra were investigated.

The human preparations ($n=84$) were taken from 16 patients, 15 undergoing cystourethrectomy because of bladder malignancy, and in one case, post mortem immediately after bilateral donornephrectomy for kidney transplantation. Thirteen of the patients were men with a mean age of 59 years (range 45–74 years). The mean age of the 3 female patients was 60 years (range 40–71 years). Twelve of the patients had received radiotherapy, in a dose of 20 Grey (2000 rad) against the bladder the week before operation. In men, preparations were taken from the prostatic ($n=26$) and from the infracollicular-membranous parts ($n=40$) of the urethra.

In women, preparations ($n=18$) were taken from the middle third of the urethra. All specimens were dissected free from adjacent mucosa and cut into preparations with a similar size and appearance as those from the rabbit.

Perfusion

The experimental preparation thus obtained consisted of 4 interconnecting strips which were rinsed in Krebs solution for 3×10 min. The preparation was placed in the perfusion chamber, and rinsed at a flow rate of 3.8 ml/min for additional 60 min with Krebs solution at a pH of approximately 7.4. The perfusion chamber was built in plexiglass, had a volume of 0.4 ml and was surrounded by a water mantle kept at 37°C. The perfusate entered the chamber at the bottom and left at the top. Two circular electrodes, each covered by a net of stainless steel, were placed at the top and under a 4 mm high plastic tube (diameter 9 mm) reducing the effective volume of the chamber to 0.2 ml. The preparation was placed within the tube.

During the experiment, the flow through the chamber was determined by a Peristaltic Pump P3 (Pharmacia, Sweden), and set at 3.8 ml/min. The temperature of the solution in the chamber was 37°C and the pH approximately 7.4. Fractions of 5.7 ml superfusate were collected in 90 s periods.

Electrical stimulation

Electrical field stimulation was performed by means of a Grass S 48 stimulator. The electrodes of two perfusion chambers were connected in series with the stimulator. Both the current strength and the voltage were displayed on an oscilloscope and the voltage producing a current strength of 150 mA was chosen (approximately 30 V over the electrodes). Single square wave pulses with a frequency of 10 Hz and a duration of 0.8 ms were delivered in trains of 60 s. The polarity of the electrodes was changed after each pulse by means of a separate polarity changing unit.

In a separate series of experiments it was secured that the frequency used produced a reproducible and submaximum degree of efflux of ^3H . In experiments with β -adrenoceptor active drugs an additional frequency of 5 Hz was used. Electrical stimulation was performed in the 7th, 22nd, 37th and 52nd min after the beginning of the fractional collection, corresponding to vials number 5, 15, 25 and 35 out of 45 vials used in one experiment.

Counting

To each vial 10 ml of a scintillation liquid (Lumagel SB, Beckman) was added and for counting a liquid scintillation spectrometer (RackBeta, LKB, Sweden) was used. After weighing, the urethral tissue was dissolved in Soluene (Packard) 2 ml at 60°C for one hour before addition of 10 ml scintillation liquid. The efficiency of the counting was 26%. Quenching curves were constructed for the solutions used and the differences found were compensated for in the analysis of the results.

Solutions

The Krebs solution had the following composition (mM): NaCl 119, KCl 4.6, CaCl_2 1.5, MgCl_2 1.2, NaHCO_3 15, NaH_2PO_4 1.2, glucose 11.0. Ascorbic acid, 0.3mM, was added to all solutions used in order to minimize the degree of breakdown of NA.

All solutions were made on the day of the experiment. They were kept in a thermostatically controlled water bath at 37°C and bubbled with a mixture of 5% CO_2 and 95% O_2 to give a pH of approximately 7.4. The different solutions used in one experiment could be interchanged within 2 s.

Drugs

The following substances were used: phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), clonidine hydrochloride (Boehringer), rauwolscine hydrochloride (Carl Roth), isoprenaline hydrochloride (Riker Laboratories), dl-propranolol, d-propranolol (ICI), carbamylcholine-chloride, (BDH Chemicals Ltd), atropine sulphate (ACO), scopolamine hydrochloride, nicotine amide, hexamethoniumbromide, hemicholine-3 (Sigma),, mepivacaine hydrochloride (Astra) and tetrodotoxin (Sigma).

All substances were dissolved in Krebs solution containing ascorbic acid. The contact time for all drugs was 13 min. In the experiments with hemicholine the preparations were exposed to the drug for 1 h after the two first stimulation periods, S_I and S_{II} . Hemicholine was then also present during the second half of the experiment. An untreated preparation, run in parallel, served as control in these experiments.

Of the four stimulations ($S_I - S_{IV}$) performed in one experiment the mean of the two first were used as controls. The drugs were added in different concentrations before the 3rd and 4th stimulation. The lower concentration was always given first.

Calculations and statistical analysis

The fractional release was defined as the electrically induced efflux of ^3H after one stimulation period in relation to all residual ^3H at the time of onset of stimulation. The fractional release in $S_I - S_{IV}$ was calculated as the efflux of ^3H in the first three fractions following each stimulation, corresponding to a period of 4,5 min, the stimulation period included. All results are given in % of the fractional release in S_{III} or S_{IV} in a series of control experiments ($n=12$). The stimulation induced efflux in S_{III} and S_{IV} in the control experiments was set to 100%. Statistical analysis was performed by means of Student's t-test for paired and unpaired data, when appropriate. Statistical analysis of the results obtained from experiments with human tissue was performed only for the male urethra due to the small number of observations on preparations from the female urethra. $P < 0.05$ was regarded as significant. Results are given as mean $\pm$ standard error of the mean, unless otherwise stated.

Methodological considerations

By means of high pressure liquid chromatography (HPLC) with electrochemical detection the amount of electrically induced release of ^3H from rabbit urethral preparations was shown to consist of: ^3H -noradrenaline 47%, normetanephrine 8% and deaminated metabolites 43%. The amount of ^3H -NA of the spontaneous efflux of ^3H was 3%, while the amount of ^3H -NA in the urethral preparations comprised 91% of the total amount of ^3H . In the presence of cocaine 10^{-5}M the uptake of ^3H -NA was inhibited by 83%, indicating that the electrically evoked release of ^3H was a release from adrenergic nerves.

Results

Adrenoceptors in rabbit urethra

The stimulation-induced efflux of ^3H in the control experiments ($n=12$) was in S_{II} 99 ± 2 , S_{III} 96 ± 2 and in S_{IV} $96 \pm 2\%$ of that in S_I (Fig. 1). The size of S_I

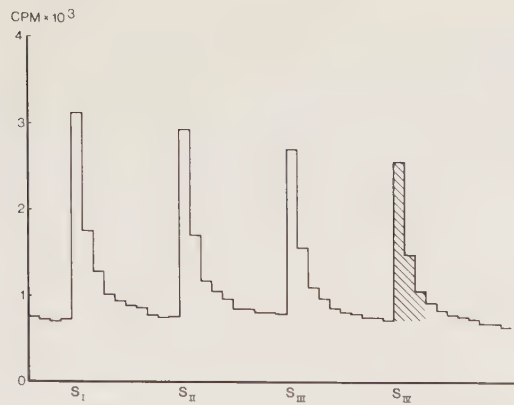


Fig 1. Electrically induced (S_I – S_{IV}) efflux of ^3H from a female rabbit urethral smooth muscle preparation preincubated with ^3H -NA. The shadowed area illustrates the amount of activity (counts per minute, CPM) used for calculation of the efflux induced by stimulation in S_{IV} .

comprised $1.4 \pm 0.07\%$ of the total uptake of ^3H in the preparation. The amount of released ^3H -NA at each stimulation varied between 1–2.5 pmoles. Tetrodotoxin 10^{-6}M abolished the stimulation induced efflux of ^3H ($n=4$).

Alpha-adrenoceptor-agonists and antagonists. The α_2 -adrenoceptor agonist clonidine caused a concentration dependent decrease of the efflux of ^3H in comparison to the controls (Figs. 2 and 3). At the concentrations 10^{-9} , 10^{-8} , 10^{-7} and 10^{-6}M ($n=6$ at all concentrations) the released fractions were 93 ± 4 , 86 ± 3 , 81 ± 2 and $67 \pm 4\%$ of the corresponding fractions in the control group. The decrease was found to be significant for clonidine 10^{-8} ($p < 0.05$), 10^{-7} ($p < 0.001$) and 10^{-6}M ($p < 0.001$).

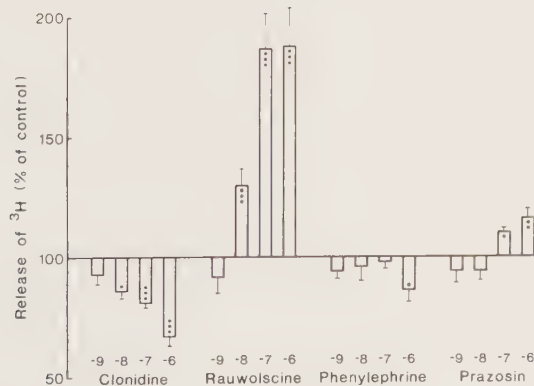


Fig 2. Changes of the electrically induced efflux of ^3H from female rabbit urethral preparations in the presence of the α_2 -adrenoceptor stimulating- and blocking agents clonidine and rauwolscine and the α_1 -adrenoceptor stimulating- and blocking agents phenylephrine and prazosin. The results are expressed in % of a series of control experiments ($n=12$). Bars indicate standard error of the mean. The concentrations used were 10^{-9} – 10^{-6}M . $n=6$ at all concentrations. $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$

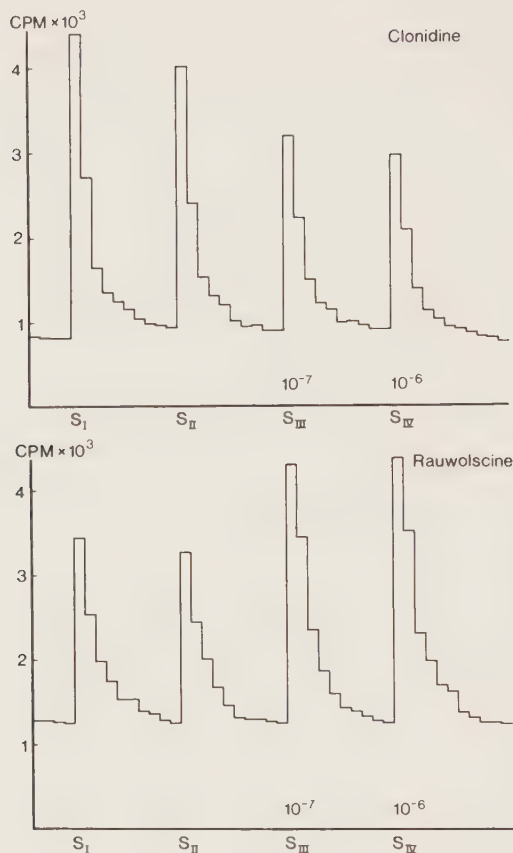


Fig 3. The influence of the α_2 -adrenoceptor stimulating and blocking agents clonidine and rauwolscline 10^{-7} and 10^{-6} M on the electrically induced release of ^3H from female rabbit urethral smooth muscle preparations preincubated with ^3H -NA.

The α_2 -adrenoceptor antagonist rauwolscline in the concentrations 10^{-8} , 10^{-7} and 10^{-6} M ($n=6$ at all concentrations) significantly increased the stimulation induced efflux of ^3H (Figs. 2 and 3). The stimulation induced efflux was 130 ± 7 , 187 ± 15 and $188 \pm 16\%$, respectively, of the efflux in the control group. These changes were significant ($p < 0.001$). Rauwolscline in a concentration of 10^{-9} M ($n=6$) had no significant effect.

At the concentrations 10^{-9} and 10^{-8} M the α_1 -adrenoceptor-stimulating and -blocking agents phenylephrine and prazosin did not cause any significant changes of the stimulation induced efflux of ^3H . However, at the highest concentration used, 10^{-6} M, phenylephrine decreased the ^3H -efflux to $86 \pm 5\%$ ($n=6$, $p < 0.05$) whereas prazosin 10^{-7} and 10^{-6} M caused an increase to 110 ± 2 ($n=6$, $p < 0.05$) and $116 \pm 4\%$ ($n=6$, $p < 0.01$) of the controls (Fig. 2). In the presence of phenylephrine 10^{-7} and 10^{-6} M there was an increase of the basal level of the efflux of ^3H (Fig. 4). This increase was not influenced by the presence of propranolol 10^{-6} M in the perfusion fluid.

Beta-adrenoceptor agonists and antagonists. Isoprenaline 10^{-9} ($n=4$), 10^{-8} ($n=4$), 10^{-7} ($n=6$) and 10^{-6} M ($n=6$) or propranolol 10^{-9} ($n=6$), produced no significant effects on the electrically induced efflux. An increase to $110 \pm 13\%$

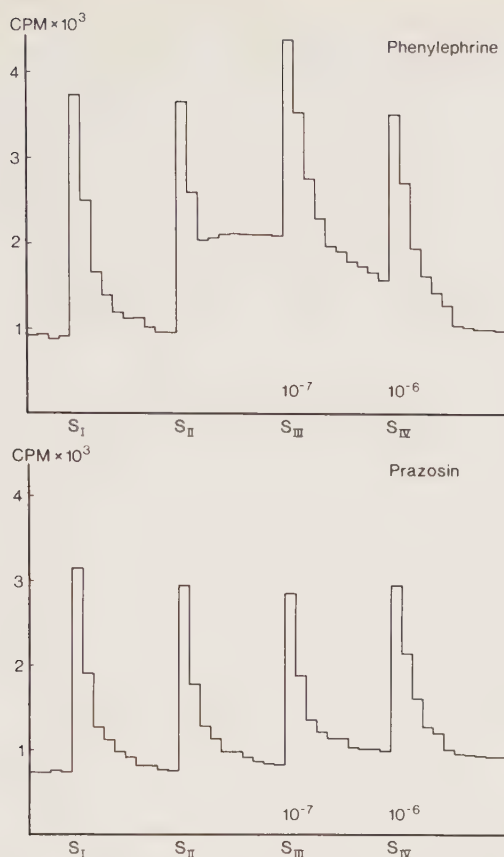


Fig 4. The influence of the α_1 -adrenoceptor stimulating and blocking agents phenylephrine and prazosin 10^{-7} – 10^{-6} M on the electrically induced release of ^3H from female rabbit urethral smooth muscle preparations preincubated with ^3H -NA.

was seen after isoprenaline 10^{-6} M (in two preparations ^3H efflux increased by 31 and 59%). At a concentration of 10^{-6} M propranolol caused a significant ($p < 0.001$) decrease to $79 \pm 4\%$ ($n=6$) of the value of the control group. Also in the concentrations 10^{-8} ($n=6$) and 10^{-7} M ($n=6$) propranolol caused a significant decrease of the efflux of ^3H to 88 ± 4 and $92 \pm 1\%$ ($p < 0.05$). The effects of the two drugs were not changed by reducing the stimulation frequency to 5Hz. Propranolol is known to have local anaesthetic as well as β -receptor blocking properties. The d-form of propranolol, 10^{-6} M, which has only about 1/100 of the β -receptor blocking potency of the racemate (11) caused a decrease of the stimulation-induced efflux of the same magnitude (to $87 \pm 4\%$ of the control, $n=6$) as the racemate at 10^{-8} M. However, no significant change of the release was seen in the presence of d-propranolol 10^{-7} M ($n=6$). The local anaesthetic mepivacaine, 10^{-7} ($n=4$) and 10^{-6} M ($n=4$), did not influence the stimulation-induced efflux of ^3H .

Adrenoceptors in human urethra

In control preparations from the female human urethra the size of the stimula-

tion induced release in S_1 was $1.8 \pm 0.3\%$ ($n=7$). The corresponding figures for the male prostatic and membraneous urethra were $1.8 \pm 0.4\%$ ($n=10$) and $1.9 \pm 0.4\%$ ($n=7$), respectively. Also in human preparations tetrodotoxin 10^{-6}M abolished the stimulation-induced efflux of ^3H .

In the male human urethra the α_2 -adrenoceptor stimulating and blocking agents clonidine and rauwolscline caused significant changes of the stimulation-induced efflux of ^3H . These changes were of the same magnitude in both the prostatic ($n=5$) and membraneous ($n=4$) parts of the urethra. Clonidine 10^{-7} and 10^{-6}M decreased the release of ^3H to $88 \pm 2\%$ ($n=9, p<0.01$) and $75 \pm 6\%$ ($n=9, p<0.01$), respectively, while rauwolscline 10^{-7} and 10^{-6}M increased the release to $114 \pm 4\%$ ($n=10, p<0.01$) and $111 \pm 3\%$ ($n=10, p<0.01$), respectively.

In preparations from the female urethra rauwolscline 10^{-7}M ($n=3$) and 10^{-6} ($n=3$) produced an increase of the stimulation-induced efflux of ^3H to $135 \pm 4\%$ and $123 \pm 6\%$, respectively. Clonidine 10^{-7} ($n=3$) and 10^{-6}M ($n=3$) decreased the efflux of ^3H to 90 ± 7 and $74 \pm 7\%$.

The β -adrenoceptor agonist isoprenaline 10^{-7} or 10^{-6}M ($n=4$) did not change the release of ^3H in preparations from the male or female human urethra.

Cholinoceptors in rabbit urethra

Carbachol 10^{-5}M ($n=6$) significantly decreased the stimulation-induced efflux of ^3H to $81 \pm 7\%$ ($p<0.01$) (Fig. 5). At the concentrations 10^{-10} – 10^{-6}M no statistically significant effect of carbachol was observed.

Atropine 10^{-6} ($n=6$) and 10^{-5}M ($n=6$) increased the release of ^3H to 126 ± 5 and $159 \pm 12\%$, respectively (Fig. 5). Significant, but small, increases (to 110 ± 2 and $109 \pm 1\%$) were registered at the lower concentrations used, 10^{-8} ($n=6$) and 10^{-7}M ($n=6$). With scopolamine 10^{-6} ($n=8$) and 10^{-5}M ($n=8$) the increase of the stimulation induced efflux was 117 ± 2 and $120 \pm 3\%$, respectively (Fig. 5). These changes were significant ($p<0.001$).

After treatment of the preparations with hemicholine, $4 \times 10^{-5}\text{M}$ ($n=4$) for one hour, no change of the pattern of the stimulation-induced efflux of ^3H was observed. Nicotine, 10^{-5} ($n=4$) and 10^{-4}M ($n=4$) and hexamethonium 10^{-6} ($n=4$) and 10^{-5}M ($n=4$) had no significant effects on the stimulation-induced efflux.

Cholinoceptors in human urethra

Scopolamine 10^{-6} and 10^{-5}M increased the electrically induced efflux of ^3H in three preparations from the human female urethra to $111 \pm 10\%$ and $113 \pm 15\%$ of the controls, while a decrease to 48% was seen in one preparation after carbachol 10^{-5}M . Also in preparations from the male urethra ($n=8$) a decrease of the release of ^3H to $60 \pm 7\%$ and $67 \pm 9\%$ was seen after carbachol 10^{-6} and 10^{-5}M , respectively. These changes were significant with $p<0.001$ and $p<0.01$, respectively. Also acetylcholine 10^{-5}M ($n=3$) induced a decrease of the release of ^3H in preparations from the prostatic infracollicular and membraneous parts of the urethra of a magnitude similar to that induced by carbachol. Although the changes induced by carbachol were of similar size in both the prostatic and infracollicular-membraneous parts of the urethra the effects of scopolamine was found to differ in the two parts of the organ.

An increase to $111 \pm 4\%$ ($n=4$) and $119 \pm 6\%$ ($n=4$) was found in the prostatic part after scopolamine 10^{-6} and 10^{-5}M , while no changes in comparison to the controls were found in the infracollicular-membraneous part of the urethra.

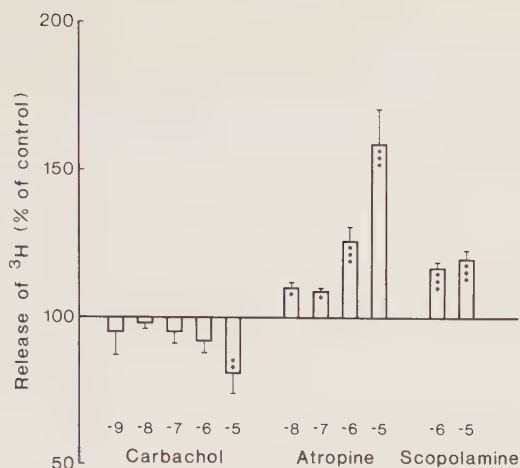


Fig 5. Changes of the electrically induced efflux of ^3H from female rabbit urethral smooth muscle preparations in the presence of the cholinceptor stimulating agent carbachol (10^{-9} – 10^{-5}M) and the muscarinic receptor blocking agents atropine (10^{-8} – 10^{-5}M) and scopolamine (10^{-6}M – 10^{-5}M). The results are expressed in % of a series of control experiments ($n=12$). Bars indicate standard error of the mean. $n=6-8$. $p<0.05=*$, $p<0.01=**$, $p<0.001=***$.

Discussion

The present results suggest the presence of α_2 -adrenoceptors and muscarinic cholinceptors located prejunctionally on noradrenergic nerve terminals in the urethra of rabbit and man, whereas no evidence for the occurrence of prejunctional α_1 -adrenoceptors or nicotinic receptors was found.

α_2 -adrenoceptors

Prejunctionally located α_2 -adrenoceptors are believed to mediate a negative feed-back regulation of the release of noradrenaline from adrenergic nerve terminals, and of acetylcholine from cholinergic nerve endings, i.e. in the intestine (12, 13, 14). These receptors are activated when the concentration of noradrenaline in the neuromuscular junction has reached a certain level (15). The observed effects of clonidine and rauwolscine in the present study were of magnitudes similar to those reported in investigations of other tissues (15). It therefore seems possible that the degree of sympathetic influence on the urethra can be modulated presynaptically. Nordling et al. (16) showed that clonidine decreased the intraurethral pressure in women. Even if this was attributed to an effect of the drug on the central nervous system decreasing the sympathetic outflow, it cannot be excluded that a peripheral, prejunctional effect of clonidine on adrenergic nerves caused a decreased NA release contributing to the decrease in intraurethral pressure.

α_1 -adrenoceptors

In agreement with the findings in previous studies on the release of ^3H -NA from adrenergic nerves (12) no evidence for the presence of prejunctional α_1 -adrenoceptors was found in the present investigation. The decrease of the stimulation-induced efflux in the presence of phenylephrine and the increase

by prazosin were caused by high concentrations of the drugs. The selectivity of the drugs in these concentrations may be questioned. Therefore it cannot be excluded that these effects were mediated via α_2 -adrenoceptors. The increase of the basal efflux of ^3H seen after phenylephrine 10^{-7} and 10^{-6}M may be caused by a NA-releasing effect of the drug (17).

β -adrenoceptors

In several tissues prejunctional β -adrenoceptors mediating a positive feed-back regulation of the release of noradrenaline have been demonstrated (18, 19, 20). The influence of these receptors are thought to dominate at a low rate of firing of the adrenergic nerves (15). In the present investigation, the β -adrenoceptor agonist isoprenaline did not cause any significant increase in the release of ^3H . However, the decrease of the stimulation-induced efflux caused by propranolol 10^{-8}M in the rabbit urethral preparations was also seen at a concentration of 10^{-6}M of the d-form of the substance, whose β -receptor blocking potency is only approximately 1/100 of that of the racemate (11). D-propranolol 10^{-7}M had no effect. Probably, these effects of propranolol were caused by blockade of β -receptors.

Propranolol has local anaesthetic properties in the same concentration range as have, e.g., lidocaine and mepivacaine (21). These properties were probably not responsible for the decrease of ^3H efflux since the local anaesthetic mepivacaine was ineffective.

Cholinoceptors

Both morphological and functional investigations indicate the presence of an interaction at a ganglionic level between adrenergic and cholinergic nerves supplying the lower urinary tract (7, 9, 22, 23). In the present investigation the possibility of an interaction at the level of the peripheral nerve terminals was investigated. Both atropine 10^{-8} – 10^{-5}M and scopolamine 10^{-6} and 10^{-5}M caused significant increases of the stimulation-induced efflux ^3H , while carbachol 10^{-5}M was found to significantly decrease the release of ^3H in preparations from both rabbit and man. The effect of carbachol at low concentrations was also investigated because facilitation of the release of ^3H -NA from adrenergic nerves has been reported to occur at low and inhibition at high concentrations of cholinceptor-stimulating agents (24, 25, 26). In the present investigation no significant changes were found after carbachol $\leq 10^{-6}\text{M}$.

The increase of the stimulation-induced efflux seen after scopolamine was considerably lower than that induced by atropine in equimolar concentrations. This difference can probably be attributed to the α -adrenoceptor blocking properties of atropine demonstrated in the rabbit urethra for concentrations exceeding 10^{-6}M using both functional and radioligand binding techniques. Scopolamine, however, did not interact with the α -receptors (27).

Nicotinic cholinoceptors are known to occur prejunctionally on adrenergic nerve terminals in some organs and there mediate facilitation of the release of noradrenaline (14, 28). Therefore, the effects of nicotine amide and hexamethonium were investigated. However, this type of cholinceptor seems to have little importance in the rabbit urethra since both drugs lacked effect on the stimulation-induced efflux of ^3H .

Human urethra

In both the male and female human urethra the effects of adrenoceptor- and

cholinoceptor-active drugs were similar to those found in the female rabbit urethra. In the male urethra α_2 -adrenoceptors and muscarinic cholinoceptors located on adrenergic nerves could be demonstrated both in the prostatic and infracollicular parts of the organ.

Although the functional importance of prejunctional adreno- and cholinoceptors *in vivo* is not necessarily proportional to the effects they are able to mediate *in vitro*, it cannot be excluded that the changes of the release of ^3H induced by α_2 - and muscarinic receptor-active drugs in the urethra, do reflect regulatory mechanisms of significance also *in vivo*. Whether disturbances in the function of these receptors play a role in the pathophysiology of the lower urinary tract remains to be established.

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IV

Effects of Vasoactive Intestinal Polypeptide on Isolated Urethral and Urinary Bladder Smooth Muscle from Rabbit and Man

C. Sjögren, K.-E. Andersson and A. Mattiasson

The Departments of Clinical Pharmacology and Urology, University Hospital of Lund, Lund, Sweden, and the Department of Obstetrics and Gynaecology, Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada.

Abstract

Vasoactive intestinal polypeptide (VIP) concentration-dependently inhibited the contractant responses of isolated preparations of the female rabbit bladder and urethra induced by electrical field stimulation and exogenous application of acetylcholine (ACh, bladder) and noradrenaline (NA, urethra). The inhibition of α -adrenoceptor and muscarinic cholinergic-mediated activity in the urethra and bladder amounted to 50–90% of induced contractions. The non-adrenergic non-cholinergic contraction induced by electrical field stimulation in the urethra was reduced slightly whereas corresponding response in the bladder was more sensitive. The maximum inhibition of both the electrically evoked responses and contractions induced by exogenous NA and ACh was of comparable size in the urethra and the bladder. The effects of VIP seemed to be exerted postjunctionally since no significant influence of the peptide was seen on the release of ^3H -NA from adrenergic nerve-endings in the urethra. The effects of VIP in human urethral and bladder preparations were less consistent. The NA-induced contraction in urethral preparations was inhibited by $29 \pm 9\%$ ($n=22$). The effects on electrically induced contractions in the urethra, and on responses to ACh and electrical field stimulation in the bladder were small and inconsistent.

It is concluded that VIP may be of importance for regulation of lower urinary tract smooth muscle activity in the rabbit. It cannot be excluded that the peptide has a modulatory role in the neurotransmission in human urethral muscle. However, the present results do not support the view of VIP being an inhibitor of contraction in human detrusor.

Introduction

In most mammals, there is a non-adrenergic, non-cholinergic nervous component involved in contraction of urinary bladder smooth muscle (1). A non-adrenergic, non-cholinergic relaxation has been demonstrated in urethral smooth muscle from man, rabbit and pig (2, 3). These responses are believed to be mediated by transmitters, the nature of which is unknown, but among the various candidates suggested (4) are peptides demonstrated to occur in the lower urinary tract of most species investigated (5, 6, 7, 8, 9). One of these is

vasoactive intestinal polypeptide (VIP), which in vascular (10, 11) as well as in other types of smooth muscle (12, 13) has been shown to have a potent relaxant effect. The actions of VIP on the lower urinary tract have not been established. Even if a relaxant effect might be expected and has been reported on the smooth muscle of both bladder (14,3) and urethra (14,2), contractant effects were found in bladder muscle from guinea pig (15) and man (16).

In the present study, the effects of VIP were investigated on isolated smooth muscle from the bladder and urethra of rabbit and man. In addition, we studied the possible modulatory effect of VIP on electrically induced release of noradrenaline from adrenergic nerves in isolated rabbit urethral muscle.

Material and methods

Rabbit

Female rabbits with an average weight of about 3 kg were stunned by a blow on the head and exsanguinated. The bladder and urethra were immediately dissected free down to the entrance of the vaginal wall. The bladder was opened and the trigonal area was identified. Two circular transverse sections, each approximately 4 mm long, were taken from the upper and middle parts of the urethra. The ring-formed preparations were transferred to a 10 ml temperature-controlled organ bath filled with Krebs solution (composition, see below) that was maintained at 37°C and bubbled with a mixture of 95% O₂ and 5% CO₂ to give a pH of 7.4. The preparations were mounted between two L-shaped platinum hooks within the lumen of the urethral ring. The upper hook was connected to a force transducer (Statham FT 03) for registration of isometric tension. The lower hook, fixed to a movable unit allowing adjustments of tension served as an electrode for electrical stimulation. The other platinum electrode was S-shaped and placed close to the preparation. The urethral rings were stretched repeatedly and were then allowed to relax until a stable tension level of approximately 8 mN was obtained. The equilibration period was 60 min.

The urinary bladder was separated by a transverse cut above the trigone. After removal of the mucosa, detrusor strips (6–10 mm long and 2 mm wide) were prepared and suspended between two platinum electrodes. Isometric tension was registered by force transducers (Grass Instrument FT03) and the basal tension was adjusted to about 5 mN.

Man

Urethral and/or detrusor preparations were obtained from 20 patients (9 females aged 34–67 years, and 11 males, aged 56–72 years) undergoing cystourethrectomy *en bloc* because of bladder cancer, or open cystostomy. Radiologic treatment against the bladder was given to 9 of these patients in a dose of 20 Grey (2000 rad) during the week before surgery. All tissue specimens appeared macroscopically normal with no signs of tumor or inflammation.

Rings of tissue were taken from the zones representing the maximum urethral pressure, i.e. the infracollicular part of the prostatic urethra and the membranous urethra, and from the middle part of the female urethras. The rings were opened, the mucosa and submucosa removed, and the underlying tissue carefully dissected into strip preparations approximately 1–2 mm wide, 0.5–1 mm thick and 8–10 mm long.

Tissue specimens were taken from the fundus of the bladder. The mucosa was removed and strips (approximately 1–2 mm wide and thick and 10 mm long) were dissected in different directions.

Both urethral and bladder preparations were mounted in thermostatically controlled organ baths filled with Krebs solution kept at 37°C and bubbled with a mixture of 95% O₂ and 5% CO₂. Isometric tension was recorded by means of Grass Instrument FT03 force transducers connected to a Grass model 7D polygraph. The preparations were allowed an equilibration period of 1–1.5 h. They were repeatedly stretched until a stable tension was obtained. Tension was then adjusted to about 8 mN for urethral and 5 mN for bladder preparations. Before accepting a preparation for experiments, its contraction capacity was tested by exposure to noradrenaline (NA, 8 and 60 µM, urethra) and acetylcholine (ACh, 50 µM, bladder). Only strips responding with reproducible contractions (less than 10% variation) were accepted for the study.

Electrical stimulation

The current used for electrical stimulation was delivered by a Grass stimulator, model S48. The preparations were stimulated by single square wave pulses of 0.8 ms duration at a frequency of 1–50 Hz. The voltage was supramaximum, train duration 5 s, and the stimulation interval 2 min.

Noradrenaline release

The effects of VIP on the release of NA from adrenergic nerve endings was investigated by studying the release of ³H from female rabbit urethral preparations preincubated with ³H-NA (14.2Ci/mM, New England Nuclear). The method used has been described in detail previously (17).

One of two preparations run in parallel was exposed to VIP 5×10^{-7} M for 10 min before the third (S_{III}) of four (S_I–S_{IV}) stimulation periods. The other preparation served as control. The electrically induced efflux of ³H in the four stimulation periods was expressed as a fraction of the total amount of activity in the preparation at the time of onset of stimulation. In the VIP experiments, the size of the released fraction of ³H in S_{III}, was expressed in relation to the size of S_{III} in the control experiments.

Student's t-test for paired data was used for statistical analysis.

Solutions

The Krebs solution used had the following composition (mM): NaCl 118, KCl 4.6, CaCl₂ 1.5, MgSO₄ 1.15, NaHCO₃ 24.9, KH₂PO₄ 1.4, glucose 5.5. In the release experiments 0.3 mM ascorbic acid was added to the Krebs solution in order to stabilize the NA in the solution.

Drugs

Acetylcholine chloride, atropine sulphate, isoprenaline sulphate, noradrenaline hydrochloride, phentolamine mesylate, propranolol hydrochloride, scopolamine hydrochloride, vasoactive intestinal polypeptide, VIP (Sigma).

Results

Rabbit

Urethra. NA induced a reproducible and concentration-related contraction in the urethral ring preparations. Maximum contractile force was reached at a concentration of $6 \times 10^{-5} \text{M}$. Addition of VIP in the concentration range $5 \times 10^{-9} - 10^{-6} \text{M}$, 2–4 min before contractions were induced by NA in a concentration ($3 \times 10^{-7} \text{M}$) producing a mean of 31% (range 22–40%) of maximum contraction, reduced the NA response in a concentration related manner (Fig 1). VIP $5 \times 10^{-9} \text{M}$ did not significantly decrease the NA response ($n=9$),

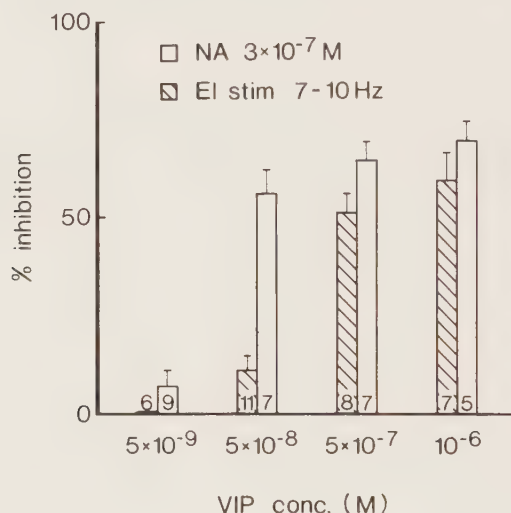


Fig 1. The inhibitory effect of vasoactive intestinal polypeptide (VIP) on contractant responses of female rabbit urethral ring preparations (mean $\pm$ SE). Contractions were induced by exogenous application of noradrenaline (NA $3 \times 10^{-7} \text{M}$) and submaximum frequency (7–10 Hz) electrical field stimulation. The number of experiments are given within the bars.



Fig 2. The effects of vasoactive intestinal polypeptide (VIP) on a ring preparation from a female rabbit urethra contracted by means of noradrenaline (NA).

but at a concentration of $5 \times 10^{-7} \text{M}$, the peptide caused an inhibition of $64 \pm 5\%$ ($n=7$). When using NA $5 \times 10^{-6} \text{M}$ (producing a contraction about 75% of maximum), the inhibitory effect of VIP $5 \times 10^{-7} \text{M}$ was $71 \pm 5\%$ ($n=7$). There was no difference in the reaction to VIP between the proximal and distal parts of the urethra. In some preparations ($n=5$) VIP $5 \times 10^{-7} \text{M}$ was added when the NA ($3 \times 10^{-7} \text{M}$) induced contraction had reached its maximum. The tension of the preparation then fell to the basal level (Fig 2).

Electrical stimulation induced a frequency-dependent contraction of the urethral preparations (Fig 3). These contractions could be separated into two main components, one initial, fast developing, resistant to anticholinergic drugs and alpha-adrenoceptor blockers, and a second slowly developing part, sensitive to alpha-adrenoceptor blockade (18). Maximum contractile force was reached at a frequency of 40–50 Hz. Submaximum frequencies (7–10 Hz, producing contractions approximately 20% of maximum) were used for studying the effect of VIP (Fig 1). It was shown that VIP $5 \times 10^{-9} - 10^{-6} \text{M}$ caused a concentration related inhibition of the contractions. VIP $5 \times 10^{-9} \text{M}$ ($n=6$), had no effect, but at a concentration of $5 \times 10^{-7} \text{M}$ the inhibition was $52 \pm 4\%$ ($n=8$). Contractions induced by higher frequencies, (25–30 Hz, producing 70–80% of maximum contraction) were inhibited by VIP $5 \times 10^{-7} \text{M}$ by $59 \pm 7\%$ ($n=6$). The maximum effect was generally reached within 10 min.

Like phentolamine VIP preferentially inhibited the alpha-adrenoceptor-blocker sensitive, second part of the electrically induced contraction (Fig 3). The first part of the response was inhibited by phentolamine 10^{-6}M and VIP $5 \times 10^{-7} \text{M}$ by $25 \pm 3\%$ ($n=5$) and $20 \pm 4\%$ ($n=5$), respectively. Corresponding figures for the second part were $68 \pm 5\%$ ($n=5$) and $87 \pm 4\%$ ($n=8$).

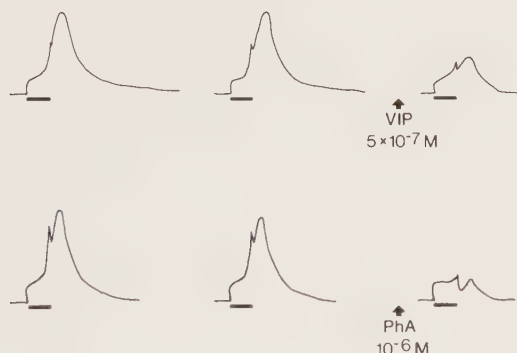


Fig 3. The effects of vasoactive intestinal polypeptide (VIP) and phentolamine (PhA) on the electrically induced contractant response of isolated female rabbit urethral ring preparations. The interval between the two first complexes in each recording was 2 min and between the second and third complexes 10 min. Bars indicate stimulation train = 5 s. Stimulation frequency 30 Hz.

Bladder. ACh in a concentration of $5 \times 10^{-6} \text{M}$ induced reproducible contractions (30–40% of maximum) in the rabbit detrusor strips. These contractions were inhibited by VIP $5 \times 10^{-9} - 10^{-6} \text{M}$ in a concentration-related way (Fig 4). VIP $5 \times 10^{-9} \text{M}$, added 2–4 min prior to ACh had a small effect ($5 \pm 3\%$; $n=6$), whereas a concentration of $5 \times 10^{-7} \text{M}$ inhibited the contractile response by $60 \pm 3\%$ ($n=6$). In experiments where contractions were induced by ACh

$5 \times 10^{-5} \text{ M}$ (producing 70–80% of maximum), the effects of VIP 5×10^{-7} were of similar magnitude being $56 \pm 6\%$. Electrically induced contractions in bladder strips evoked by 10 Hz (30–40% of maximum) were also inhibited by VIP (Fig 4 and 5). A concentration of $5 \times 10^{-9} \text{ M}$ had no effect, whereas $5 \times 10^{-7} \text{ M}$ caused an inhibition of $55 \pm 6\%$ ($n=7$). Contractions evoked by 25 Hz (70–80% of maximum) were inhibited by VIP $5 \times 10^{-7} \text{ M}$ by $58 \pm 6\%$ ($n=6$).

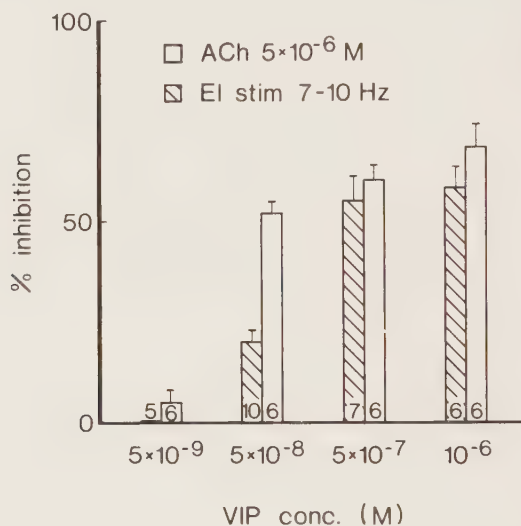


Fig 4. The inhibitory effect of vasoactive intestinal polypeptide (VIP) on contractant responses of strip preparations of the rabbit detrusor (mean $\pm$ SE). Contractions were induced by exogenously applied acetylcholine (ACh $5 \times 10^{-6} \text{ M}$) and electrical field stimulation (7–10 Hz). The number of experiments are given within the bars.

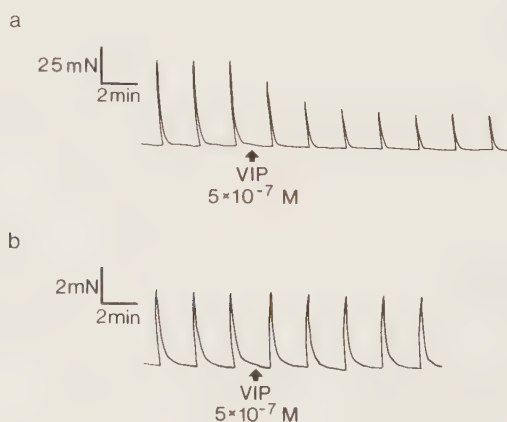


Fig 5. Vasoactive intestinal polypeptide (VIP) added to isolated rabbit (a) and human (b) detrusor strip preparations subjected to electrical field stimulation. Stimulation frequency 30 Hz.

Isoprenaline 10^{-6} M inhibited the electrically induced response in these strips by $70\pm 5\%$ ($n=8$). Propranolol 10^{-6} M added 15 min before VIP 5×10^{-7} M did not influence the inhibitory effect of VIP, but completely blocked the inhibition produced by isoprenaline.

Atropine or scopolamine 10^{-6} – 10^{-5} M caused an inhibition of the electrically induced response in the rabbit urinary bladder preparations amounting to $75\pm 8\%$ ($n=6$). VIP 5×10^{-7} M added when the inhibition of the anticholinergics was maximal, produced a marked reduction of the remaining response ($93\pm 2\%$).

All effects of VIP, both on urethral and bladder tissue strips were easy to overcome by washing the preparations.

Man

The effects of VIP 5×10^{-8} – 10^{-6} M were studied on NA induced contractions (NA 5×10^{-5} M producing 70–80% of maximum, or NA added cumulatively) in urethral strips ($n=26$), and ACh induced contractions (ACh 5×10^{-5} M producing 70–80% of maximum) in detrusor strips ($n=15$). Effects of VIP (5×10^{-8} – 5×10^{-7} M) were also studied on electrically induced contractions in urethral muscle (evoked by a frequency of 20 Hz producing 20–40% of maximum) and in detrusor muscle (35 Hz producing 70–80% of maximum). It was found that VIP had no significant effect on the ACh induced contractions in the detrusor ($n=24$, from 14 patients) in the concentrations used (Fig 5). In urethral preparations, VIP 5×10^{-7} M reduced the contraction evoked by NA 5×10^{-5} M by $29\pm 9\%$ (range 0–85%, $n=22$) and also depressed the concentration response curve to NA, added cumulatively ($n=4$, Fig 6).

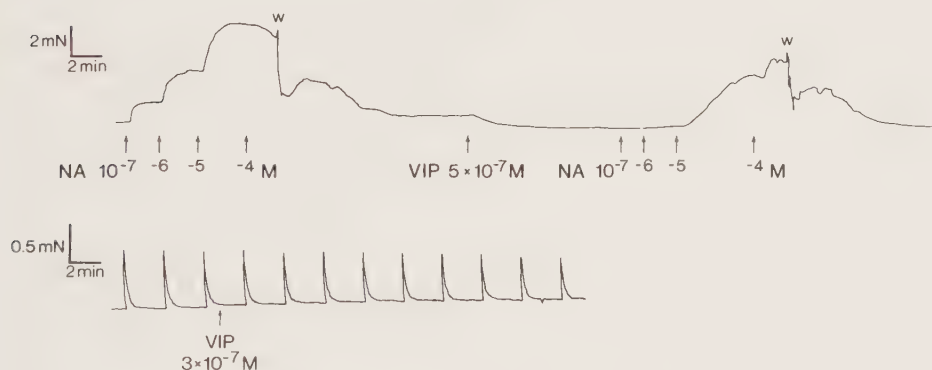


Fig 6. The influence of vasoactive intestinal polypeptide (VIP) on the concentration response-relation of noradrenaline (NA) in a female human urethral muscle preparation (upper curve) and in a preparation from the membranous part of a male urethra (lower curve) subjected to electrical field stimulation. Stimulation frequency 25 Hz.

No consistent effect was found on contractions induced by electrical stimulation neither in urethral ($n=12$) nor in detrusor preparations ($n=16$) (Figs 5 and 6).

In some bladder and urethral preparations exhibiting spontaneous contractile activity, VIP decreased both spontaneous contractions and basal tension.

Noradrenaline release

The fractional release of ^3H in the control experiments was well reproducible. In the presence of $\text{VIP } 5 \times 10^{-7}\text{M}$, no significant influence on the release of ^3H was seen. The fractional release in S_{III} in these experiments was $104 \pm 3\%$ ($n=6$) of the expected release calculated from the control experiments ($n=6$).

Discussion

VIP has been shown to have a relaxant effect in several types of smooth muscle from various species, e.g., vascular muscle from dog, rabbit and cat (10, 11, 19), rabbit uterus (13, 20), canine tracheal muscle (21), and human fallopian tube (22). In cat submandibular gland, both the increase in salivary secretion, and the vasodilatation induced by acetylcholine were potentiated by VIP (23). It was shown that the increased salivary response to acetylcholine could be attributed to VIP causing an increased affinity of ACh to the muscarinic receptors. In some parts of the body and in some species, VIP may thus have the role of a neuro-transmitter or -modulator (24).

VIP containing nerves have been demonstrated by immunocytochemical technique in the urinary bladder and urethra of several mammals including man (8, 25). Generally, the supply of VIP-ergic nerves in the human tissue was less than in, e.g., cat. The amounts of VIP have also been quantified, and were about $10 \text{ pmol} \times \text{g}^{-1}$ in the human bladder ($12\text{--}45 \text{ pmol} \times \text{g}^{-1}$ in the cat, $3.5 \text{ pmol} \times \text{g}^{-1}$ in the rat) and approximately $20 \text{ pmol} \times \text{g}^{-1}$ in the male human urethra ($56\text{--}179 \text{ pmol} \times \text{g}^{-1}$ in the cat) (26, 14, 25). However, few reports concerning the role of VIP in these organs have been published. Johns (15) observed, on addition of VIP to guinea pig urinary bladder strips, a weak contractile response and a small potentiation of the electrically induced contraction. He concluded that VIP did not fulfill any criteria for being a transmitter in the guinea pig urinary bladder. Larsen et al. (14) reported inhibitory VIP effects on isolated feline bladder strips contracted by carbachol, and on isolated feline urethra contracted by NA. A relaxant effect of VIP on isolated cat urethra could not be confirmed by Abdel-Hakim et al. (27). Reports on contractile effects on human bladder (16), and inhibitory actions on both human urethra (28) and bladder (3) have made the actions of VIP on lower urinary tract smooth muscle uncertain.

In the present study, it was found that VIP inhibited the contractile responses induced by NA in the isolated rabbit urethra and by ACh in isolated rabbit bladder strips. Inhibitory effects were also found on electrically induced contractions in both urethral and bladder muscle. In the bladder, the VIP-induced relaxation was smaller than that produced by isoprenaline. Beta-adrenoceptors did not seem to be involved in the VIP response, since propranolol did not influence the effects of the peptide. The adrenergic part of the electrically induced response in the rabbit urethra was more sensitive to VIP than the preceding non-adrenergic, non-cholinergic contraction. In the bladder, however, where the electrically induced contraction was also markedly inhibited by VIP, the response remaining after treatment with atropine or scopolamine could be almost abolished by VIP. Thus, the non-adrenergic, non-cholinergic responses in rabbit bladder and urethra seem to differ in their sensitivity to VIP. No effect on the release of noradrenaline from the adrenergic nerves of the rabbit urethra was found. This suggests that VIP has no modulatory role in the release of noradrenaline, and that its effects are exerted post-junctionally in the urethra.

The present results cannot exclude that in the rabbit lower urinary tract, VIP may have a role as neurotransmitter or neuromodulator. Such a role of the peptide has previously been suggested in the feline male genitourinary tract (14). This, on the other hand, does not seem to be the case in the human bladder when judged from the present *in vitro* data. In the bladder strips, neither ACh induced responses, nor contractions induced by electrical field stimulation were significantly affected, which is in marked contrast to the findings in the rabbit detrusor. Only occasionally a distinct inhibitory effect was seen on NA contracted urethral preparations, and there was no consistent effect on contractions induced by electrical stimulation. This difference between rabbit and human muscle cannot be attributed to the experimental conditions. It is known that peptides are lost in experimental systems because of sticking to glass and plastic materials, and that such losses may be extensive. The active concentration of peptide in the bath can therefore be considerably lower than the given. As rabbit and human tissues were tested in the same system, the same losses of peptide could be expected. Thus, it seems as if the difference in effect can be attributed to species variation, unless the experimental conditions made the human tissue less responsive. However, even if the available human lower urinary tract smooth muscle has disadvantages as experimental tissue, both bladder and urethral preparations in general are as sensitive as rabbit tissue and respond in a reproducible way to both contractant and relaxant agents (4).

Though it cannot be excluded that VIP has a role in neurotransmission in human urethral muscle, the present results do not support the view that VIP should act to modify the background activity of the detrusor muscle and keep it relaxed during the filling phase (25).

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v

Contractant and Relaxant Properties of the Female Rabbit Urethral Submucosa

Anders Mattiasson, Karl-Erik Andersson and Christer Sjögren

From the Department of Obstetrics and Gynecology, Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada, and the Departments of Clinical Pharmacology and Urology, University of Lund, Lund, Sweden

Abstract

Isolated submucosal (lamina propria) preparations from the female rabbit urethra exhibited both contractant and relaxant properties. The nerve-mediated contraction to electrical field stimulation was adrenergic in nature, and both this response and the contraction induced by exogenous application of noradrenaline (NA) was blocked to a greater extent by α_2 - than by α_1 -adrenoceptor blocking agents. Vasoactive intestinal polypeptide (VIP) was found to be a potent inhibitor of the NA-mediated contraction and neuropeptide Y (NPY) induced contraction of the preparation, but also inhibited the nerve-mediated contractant response. In NA-contracted preparations, electrical field stimulation induced a non-adrenergic, non-cholinergic relaxation. The maximum relaxant response was significantly greater when the preparations were contracted by clonidine than by NA.

An abundant occurrence of smooth muscle cells with no obvious connection to vessel walls was found in the submucosa, but to what extent the contractant and relaxant responses can be ascribed vascular or non-vascular smooth muscle is unsettled.

The results indicate a non-uniform distribution of the peripheral nervous control within the wall of the female rabbit urethra. The demonstrated contractant and relaxant properties of the submucosal tissue might be of importance for the urethral function.

Introduction

The maximum urethral pressure is an expression of active and passive forces within the distal continence-maintaining zone of the urethral wall. The active forces have been attributed to the smooth and striated muscle layers, while the passive forces result from the elastic tissue components and hydrostatic factors. Among the latter, hemodynamic influences seem to be of great importance for maintenance of the intraurethral pressure. Thus, both in dog¹ and man², approximately 30% of the maximum urethral pressure has been attributed to vascular components. A rich vascularization with large venous plexa and arteriovenous shunts is found within the submucosal layer of the urethra^{3,4,5}, and in several species, e.g. cat, dog and man, this tissue comprises a considerable part of the urethral wall^{5,6-9}. It has been suggested that the submucosal tissue together with the mucosa has passive visco-elastic properties of conti-

nence-maintaining importance, the so called "inner urethral softness"¹⁰⁻¹³. In the present investigation, which was performed to study whether the urethral submucosal tissue might also possess contractant and relaxant properties, submucosal strip preparations from the female rabbit urethra were investigated.

Material and methods

Adult female rabbits of the Danish Land and New Zealand races with an average weight of approximately 3 kg were killed by injection of air into an ear-vein. The abdomen was opened and the bladder and urethra removed *en bloc* together with the distal part of the vagina. The urethra was opened longitudinally in the dorsal wall and cut above the entrance into the vaginal wall and at the internal urethral orifice. Specimens of mucosal and submucosal tissue were taken from the longitudinal folds of the urethra. The majority of preparations were taken from the most prominent fold, "the urethral crest" situated in the twelve o'clock position. After removal of the mucosal lining, strip preparations with a size approximately 1×2×6 mm were cut from the submucosal tissue using a dissection microscope (×6). The tissue strips were transferred to 5 ml organ baths containing Krebs solution (for composition see below) kept at 37°C and bubbled with a mixture of 5% CO₂ and 95% O₂ to give a pH of 7.4. They were suspended between two L-formed hooks, one of which was movable to allow adjustments of the tension. The other hook was fixed and connected to a Grass Instruments FT03 force transducer. The "isometric tension" was recorded on a Grass Model 7D Polygraph. During an equilibration period of approximately 1 h the tension was adjusted to a final level of 3–5 mN. The contractant capacity of each preparation was characterized by the response to cumulatively added noradrenaline 10⁻⁸–10⁻⁴M.

Electrical field stimulation

Two platinum electrodes were placed close to and parallel with the preparation. Electrical stimulation was performed by means of a Grass Model S88 electrical stimulator delivering 0.8 ms single square wave pulses in trains of 5 s at supramaximum voltage. The frequency used was 0.5–50 Hz and the stimulation interval 2 or 3 min. The polarity of the electrodes was changed after each pulse by means of a polarity changing unit. The frequency producing a contraction amounting to 70–80% of the maximum response was chosen for the investigation. Three consecutive reproducible responses (accepted variation <10%) were regarded as representative.

Morphology

Preparations representing the whole urethral wall were processed for light microscopy. The preparations were taken from each of the proximal two thirds of the female rabbit urethra and sectioned in series. Transverse sections were cut, investigated in light microscope and photographed. A series of submucosal preparations (n=6) already used for functional investigations where they had shown representative responses on exposure to drugs and to electrical field stimulation, were also processed for light microscopy and photographed.

Solutions and drugs

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.6,

CaCl₂ 1.5, MgSO₄ 1.15, NaHCO₃ 24.9, KH₂PO₄ 1.4, glucose 5.5. All solutions were prepared the day of the experiment. Double distilled water was used and all chemicals were of analytical grade.

Drugs

Noradrenaline hydrochloride (NA), phenylephrine hydrochloride (Sigma), clonidine hydrochloride (Boehringer-Ingelheim), phentolamine methanesulphonate (CIBA-Geigy), prazosin hydrochloride (Pfizer), rauwolfscine hydrochloride (Carl Roth), acetylcholine chloride (ACh), scopolamine hydrochloride, vasoactive intestinal polypeptide (VIP) (Sigma), neuropeptide Y (NPY) (Peninsula Laboratories), tetrodotoxin (TTX) (Sigma). All concentrations given are final bath concentrations.

Results

Morphology

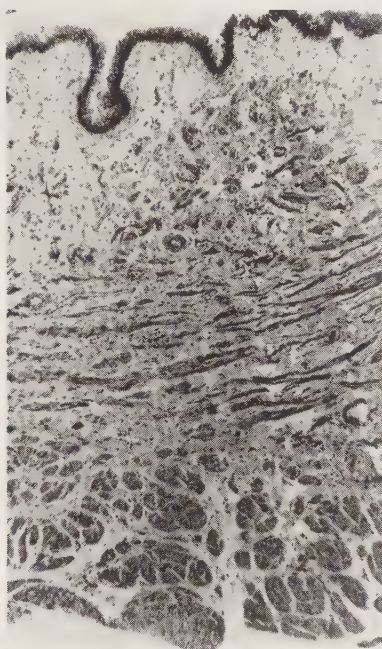


Fig 1. A transverse section through the wall of a female rabbit urethra. The submucosal layer constitutes a considerable part of the urethral wall. Hematoxylin-Eosin.

Generally a rich amount of connective tissue and smooth muscle cells were found in the submucosal tissue (fig. 1a, b). A relatively rich occurrence of vessels, preferentially veins, was also observed. Muscle cells with no obvious connection to blood vessel walls were found throughout the submucosal tissue with a condensation to longitudinal muscle bundles close to the circular muscle layer found outside the submucosa. The mucosa and submucosa formed ridges of longitudinally oriented tissue. The "urethral crest", being the most prominent of the ridges, contained tissue similar to that of the rest of the submucosa.

Light microscopic investigation of a series of preparations with representative responses on exposure to different drugs and to electrical stimulation confirmed that submucosal tissue was investigated (fig. 2).

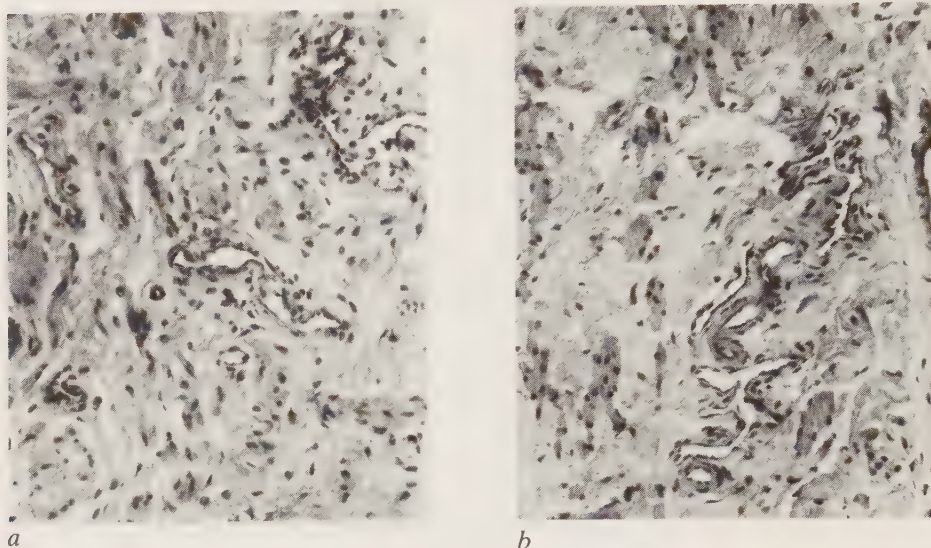


Fig 2a. Submucosal tissue in a female rabbit urethral preparation.

Fig 2b The histological picture of one of the submucosal preparations which after previous functional investigation was fixed and processed for light microscopy. Hematoxylin-Eosin.

Effects of exogenously applied drugs

Alpha-adrenoceptor active drugs

NA induced a concentration-dependent and reproducible contraction with an EC_{50} value of $10^{-6}M$ (figs. 3 and 4). The maximum response amounted to 3.0 ± 0.7 mN ($n=25$) and was reached at $10^{-4}M$. The α_1 - and α_2 -adrenoceptor stimulating agents phenylephrine and clonidine induced contractions of an amplitude similar to that of NA, but with different EC_{50} -values, $3 \times 10^{-6}M$ for phenylephrine and $9 \times 10^{-8}M$ for clonidine (fig.3). The α_1 - and α_2 -adrenoceptor blocking agents prazosin and rauwolscine inhibited the NA-induced contraction concentration-dependently. This is illustrated in fig.5, where a parallel shift to the right of the concentration response curve for NA is seen both after exposure to prazosin (fig.5a) and rauwolscine (fig. 5b).

Cholinoceptor active drugs

Acetylcholine and carbachol in concentrations of 10^{-8} – $10^{-4}M$ did not induce contraction in any submucosal preparation investigated ($n>100$). When acetylcholine $10^{-4}M$ was added to preparations contracted by a submaximum concentration of NA a relaxation of the preparations by $35 \pm 6\%$ ($n=6$) of the NA-induced tension increase was seen (fig. 6).

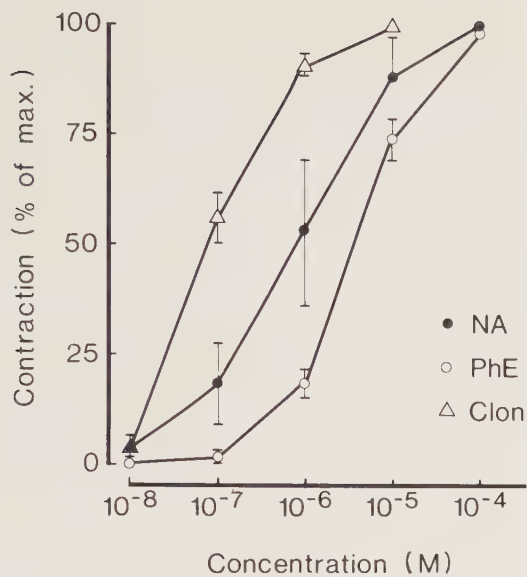


Fig 3. Concentration-response relations for noradrenaline (NA) ($n=20$), phenylephrine (PhE) ($n=7$) and clonidine (Clon) ($n=7$) in submucosal preparations from the female rabbit urethra. Mean values $\pm$ SEM are given.

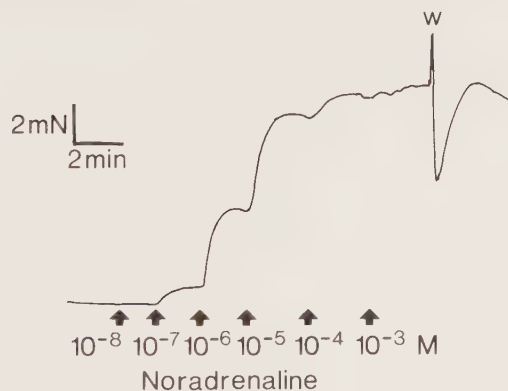


Fig 4. Concentration-response curve for noradrenaline in a submucosal preparation from the female rabbit urethra. w=wash.

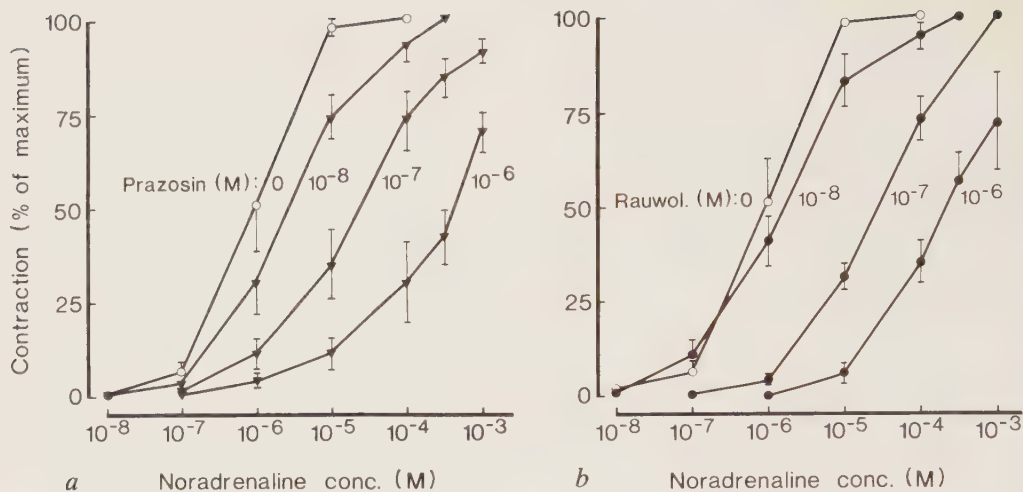


Fig 5. The effects of the α_1 -adrenoceptor blocker prazosin (a) and the α_2 -adrenoceptor blocker rauwolscine (b) on the concentration-response relations for noradrenaline in submucosal preparations from the female rabbit urethra. Maximum (100%) equals the maximum contractant response induced by noradrenaline 10^{-4} M in the absence of blocking agents (0). Mean values $\pm$ SEM, $n=6$.

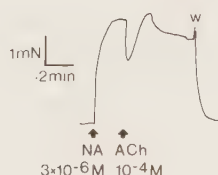


Fig 6. The relaxant effect of acetylcholine (ACh) in a noradrenaline (NA) contracted isolated submucosal preparation from the female rabbit urethra.

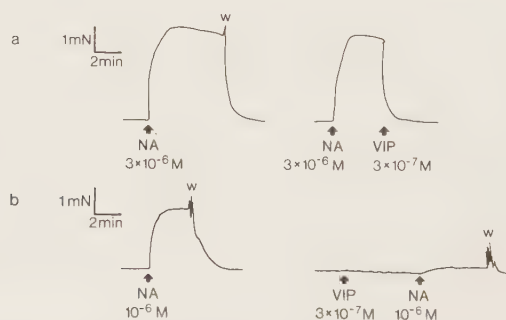


Fig 7. The inhibitory effect of vasoactive intestinal polypeptide (VIP) on noradrenaline (NA)-induced contractions in submucosal preparations from the female rabbit urethra.

Neuropeptides

VIP 3×10^{-7} M inhibited the contractant response induced by NA 10^{-6} M (EC_{50}) by $94 \pm 6\%$ ($n=6$) (fig. 7) and also produced relaxation of preparations already contracted by NA 10^{-6} M by $93 \pm 6\%$ ($n=6$) (figs. 7 and 13). The other neuropeptide investigated, NPY, induced a slight contraction (18% of the NA induced contraction) of the preparations ($n=6$) at a concentration of 10^{-7} M. In a few ($n=4$) preparations NPY 5×10^{-7} was used and a contraction amounting to $35 \pm 5\%$ of the maximum NA-induced contraction was then seen (fig. 8). The NPY-induced contractant response was not changed when phentolamine 10^{-6} M was added to the contracted preparations ($n=4$).

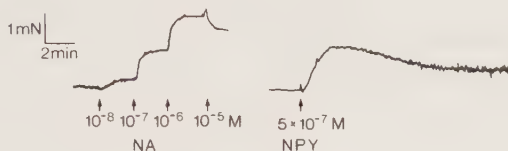


Fig 8. The contractant response to neuro peptide Y (NPY) in a submucosal preparation from the female rabbit urethra. For comparison the concentration-response curve for noradrenaline (NA) in the same preparation is shown.

Effects of electrical field stimulation

A. Contraction

Electrical field-stimulation induced a frequency-dependent contraction (fig. 9) with a threshold below 8 Hz and with maximum at 50 Hz. The typical response showed a latency of 5–15 s between the cessation of stimulation and the onset

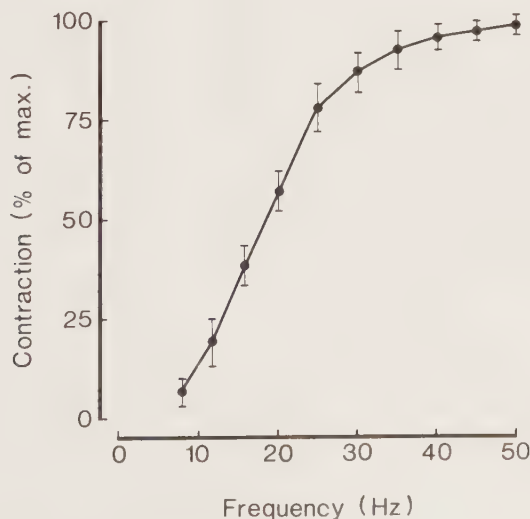


Fig 9. The frequency-response relations of the contractant response to electrical field stimulation in submucosal preparations ($n=10$) from the female rabbit urethra.

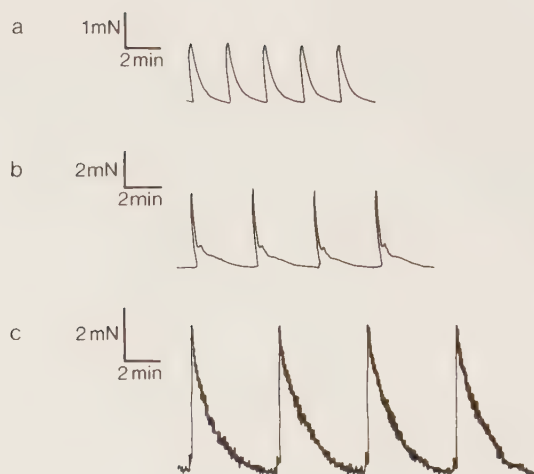


Fig 10. Different configurations of the electrically induced contractant response of submucosal preparations from the female rabbit urethra. a) the typical response found in the vast majority of preparations b) and c) less frequently recorded responses with a rapid onset of contraction.

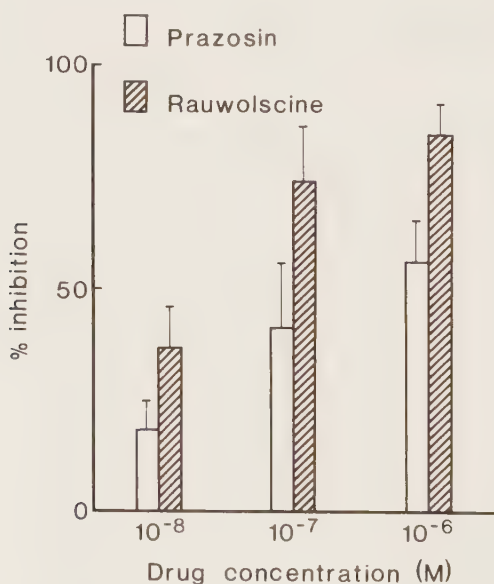


Fig 11. The inhibitory effects of α_1 - and α_2 -adrenoceptor blockade with prazosin and rauwolscline, respectively, on the electrically induced contraction of submucosal preparations from the female rabbit urethra. Mean $\pm$ SEM, $n=7-9$.

of contraction (fig. 10a). Less frequently onset of contraction was observed already during the stimulation. The configuration of the responses showed a certain variation as is shown in fig. 10b and c. In a few preparations ($n=5$) a contraction was observed during the stimulation that was not sensitive to α -adrenoceptor or muscarinic receptor blocking agents or to TTX. TTX 10^{-6} M blocked the electrically induced contractions by $96\pm3\%$ ($n=7$). In equimolar concentrations rauwolscine was a more efficient blocker than prazosin. Maximum inhibition was found at 10^{-6} M and amounted to $84\pm6\%$ ($n=6$) and $56\pm9\%$ ($n=6$), respectively (fig. 11). When both drugs were given simultaneously (concentrations 10^{-6} M) or when phentolamine 10^{-6} M was added, the response could be blocked by $96\pm4\%$ ($n=6$). Scopolamine 10^{-7} and 10^{-6} M reduced the electrically induced contractant response by $10\pm5\%$ ($n=8$) and $11\pm7\%$ ($n=8$), respectively. The neuropeptides VIP 3×10^{-7} M and NPY 3×10^{-7} M blocked the response by $94\pm4\%$ ($n=5$) (fig. 12) and $41\pm7\%$ ($n=5$), respectively.

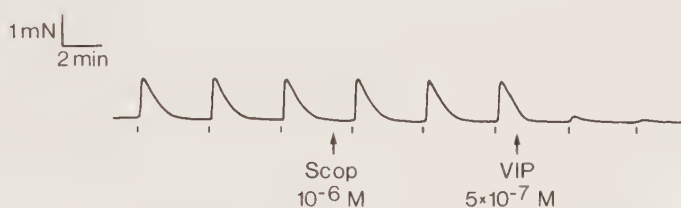


Fig 12. The effect of scopolamine 10^{-6} M and vasoactive intestinal polypeptide (VIP) 5×10^{-7} M on the electrically induced contractions of a female rabbit urethral submucosal preparation.

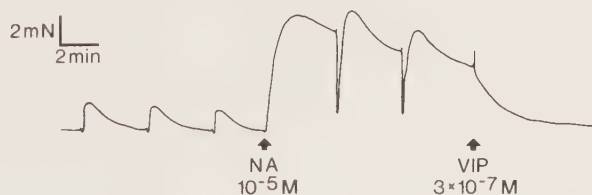


Fig 13. The responses of a submucosal preparation from the female rabbit urethra to electrical stimulation (frequency 25 Hz) at basal conditions and after contraction of the preparation induced by noradrenaline (NA). After the second relaxant response electrical stimulation was stopped. Addition of vasoactive intestinal polypeptide (VIP) induced a complete relaxation of the preparation.

B. Relaxation

After contraction of the preparations was induced by NA, the response to electrical field stimulation no longer was contraction, but relaxation (fig. 13). The relaxant response was readily blocked by TTX 10^{-6} M ($n=6$), but was unaffected by antimuscarinics and α -adrenoceptor blocking agents (except for a decrease of the NA-induced tension). The response was frequency-dependent with maximum at 10–15 Hz and with threshold below 0.5 Hz (fig. 14). The maximum relaxant response of the NA-induced contraction amounted to

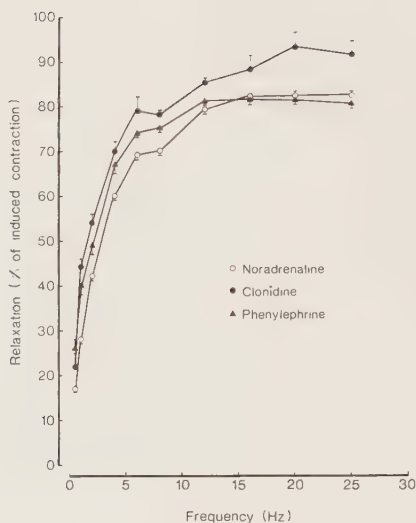


Fig 14. Frequency-response relations for the electrically induced relaxant response of submucosal preparations of the female rabbit urethra after contraction to a comparable tension level by means of submaximum concentrations of noradrenaline ($n=20$) phenylephrine ($n=9$) and clonidine ($n=11$). The maximum relaxant response of the clonidine-contracted preparations at 20 Hz differed significantly ($P<0.05$) from the relaxation induced in the same preparations contracted by noradrenaline.

$82\pm1\%$ ($n=20$), while the relaxant response after contraction of the same preparations with clonidine was significantly ($p<0.05$) greater and amounted to $94\pm3\%$ ($n=11$).

Discussion

In different species, e.g., cat, dog and man the submucosal layer of the urethra represents a considerable part of the urethral wall^{3,5-9}. According to several investigators, the submucosa of the urethra is synonymous with the lamina propria (see e.g.⁵). It is generally considered to be composed of loose connective tissue, collagen and large plexa of vessels, preferentially veins^{3,14,5}. In the morphological part of the present study, an abundant occurrence of smooth muscle cells with no obvious connection to vessel walls was found. There was a condensation of this muscular component to longitudinal muscle bundles in the deep part of the submucosa, close to the well defined circular smooth muscle layer. The amount of vascular plexa appeared to be less rich than has previously been described for urethral submucosal tissue in other species^{3,5}.

If the vascularization of the submucosal layer contributes to the maintenance of the intraurethral pressure and to urinary continence has not been established. Recording intraurethral pressure, Enhörning¹⁵ was the first to describe pulsations in the urethra synchronous with the heart beats. He concluded that the vascular component might be of importance for the urethral pressure and thus also for maintenance of urinary continence. Raz et al.¹ and Rud et al.² attributed approximately 30% of the intraurethral pressure to the vascular component. With the rich vascularization of the submucosa in mind it seems reason-

able to assume that a considerable part of the vascular influence on the urethral pressure derives from the submucosal plexus of vessels. However, Tulloch¹⁶ and Kulseng-Hanssen et al.¹⁷ in investigations on dogs questioned the role of the urethral submucosa for pressure regulation. With the photoplethysmographic method of Kulseng-Hanssen et al.¹⁷ only a filling of the human urethral venous plexa was seen at attempts of micturition. The role of the hemodynamic influence on the urethral function therefore remains to be established.

Passive, visco-elastic properties, described as the "inner urethral softness" also appear to be of importance for urinary continence¹⁰⁻¹³. It can not be excluded that the longitudinal orientation of the mucosal and submucosal folds is of functional importance, and that the soft mucosal and submucosal tissues can be regarded as a "plug" within a sheath of surrounding smooth and striated muscle.

In the functional part of the present investigation, the submucosal tissue was found to possess both contractant and relaxant properties. It could not be established to what extent these responses were caused by the smooth muscle of the blood vessel walls, and the non-vascular muscle component.

The contractant response of the submucosal preparations to electrical field stimulation was adrenergic in nature. The influence of selective α_1 - and α_2 -adrenoceptor blocking drugs on this response, as well as the effects of exogenously applied α_1 - and α_2 -adrenoceptor stimulating and blocking agents, indicated that both α_1 - and α_2 -adrenoceptors mediated the NA-induced contraction. The order of potency of clonidine-, noradrenaline- and phenylephrine-induced contractions in submucosal preparations was the same as previously has been described for whole wall rabbit urethral ring preparations¹⁸. The EC₅₀-values for clonidine did however differ slightly, being 9×10^{-8} M in the present investigation and 2.3×10^{-7} M in the investigation of ring-preparations. In submucosal tissue it seemed as if the α_2 -adrenoceptor mediated part of the response to electrical field stimulation was greater than that mediated via the α_1 -type of adrenoceptor. This is in contrast to findings in the ringformed preparations, where the α_1 -adrenoceptor mediated response was found to dominate¹⁹. These findings suggest a difference in the mode of adrenergic activation within different parts of the urethral wall, i.e., a predominance of α_2 -adrenoceptors mediating contraction in the submucosa and α_1 -adrenoceptors in other parts of the urethral wall.

In the investigations of the ring-preparations, a late and rather slowly developing contractant component was regularly seen in the contraction complex induced by electrical field stimulation¹⁹. The latency to onset of contraction observed in the present investigation corresponds well with the interval from stimulation to the start of the late contractant component in the whole wall preparation. These findings suggest that in circularly oriented whole wall preparations contraction of submucosal tissue can contribute to the response induced by electrical field stimulation.

The relaxant response in the submucosa was qualitatively comparable to that in the whole wall ring-preparations²⁰, but more pronounced with maximum relaxation amounting to $82 \pm 1\%$ of the NA induced contraction compared to $57 \pm 2\%$ in ring-preparations.

Cholinoceptor stimulating and blocking agents had little influence on the submucosa. The relaxation of the NA-contracted preparations by means of a high concentration of ACh, 10^{-4} M, might be due to the presence of inhibition-mediating muscarinic cholinergic receptors as has been described in e.g. blood vessels²¹. Since carbachol did not displace the α -adrenoceptor ligand 3 H-dihydroergocryptine from the α -adrenoceptors of the female rabbit urethra²², it

seems less likely that an influence of acetylcholine on these receptors was the cause of the relaxant effect.

Of the different neuropeptides demonstrated in the lower urinary tract, VIP and NPY are quantitatively dominating²³. Both peptides have been shown to take their origin in peripheral pelvic ganglia²⁴⁻²⁷. In the rat, a certain population of NPY containing nerve fibres, i.e. those supplying the submucosal tissue and the blood vessels of the bladder were found to be adrenergic in nature²⁷. Generally, little is known about the functional role of NPY in different organs including the lower urinary tract. In investigations of other tissues NPY was suggested to influence adrenergically and perhaps also cholinergically mediated responses. An inhibition of the electrically induced, adrenergic contraction in vas deferens was shown by Lundberg et al.²⁸, while an increase of the adrenergically mediated responses to electrostimulation was found in the gastropiploic artery of the rabbit²⁹. In vitro, NPY has also been shown to induce contraction of cerebral vessels³⁰. In the present study NPY was found to produce both direct contractant effects and inhibition of the electrically induced contraction. These effects motivate further investigations of the functional influence of NPY and of its distribution in the urethra.

The inhibitory effects of VIP on both NA-induced contractions and electrically induced responses were qualitatively comparable to that in the whole wall ring-preparation³¹, but more pronounced. This might be related to differences in the contribution of α_1 - and α_2 -adrenoceptor mediated activation between the different muscle components of the urethral wall. It cannot be excluded that VIP and NPY play a role in the neuroregulation of the contractile structures in the rabbit urethral submucosa. Whether or not VIP and NPY are neurotransmitters in the lower urinary tract will probably not be revealed until selective blocking agents are available.

Thus, the present investigation showed that the rabbit urethral submucosal tissue appears to contain both vascular and non-vascular smooth muscle cells. Not only the hydrostatic and viscoelastic properties of the submucosal tissue might be of importance for its role in urethral function, but also contractant as well as relaxant properties. Whether this is true also for the submucosal tissue of the human urethra remains to be established.

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Urethral Sensitivity to α -adrenoceptor Stimulation and Blockade in Patients with Parasympathetically Decentralized Lower Urinary Tract and in Healthy Volunteers

By

Anders Mattiasson, Karl-Erik Andersson & Christer Sjögren

Departments of Urology and Clinical Pharmacology, University Hospital, Lund, Sweden

Summary

The urethral pressure responses to i.v. infusion of increasing doses of noradrenaline (NA) and to i.v. injection of a single dose of phentolamine were compared in male and female patients with parasympathetically decentralized lower urinary tract and in normal volunteers of both sexes. Maximum urethral pressure (MUP) was significantly lower in the patients than in the volunteers. NA caused a dose-dependent increase in MUP in all subjects; the absolute increase was greater in the patients. However, even at the highest rates of NA infusion, the MUP of the patients did not reach the resting pressure of the normal volunteers, and the dose-response relations for the NA effect did not differ between patients and normal subjects. Phentolamine reduced the urethral pressure significantly less in male volunteers than in the other subjects. It is concluded that patients with parasympathetically decentralized lower urinary tract have no urethral smooth muscle supersensitivity to NA, and that it cannot be excluded that the pelvic, parasympathetic innervation contributes to the regulation of pressure in both the male and female urethra.

Introduction

In patients with parasympathetically decentralized lower urinary tract, supersensitivity of the detrusor to cholinergic agonists such as bethanechol and carbachol can often be demonstrated (Lapides et al. 1962, Glahn 1970). In such patients, urethral "supersensitivity" to α -adrenoceptor agonists has also been suggested (Koyanagi 1978). This suggestion was based on the finding of a greater increase of the maximum urethral pressure (Δ MUP) in response to a single dose of α -adrenoceptor stimulating agents in patients with lower motor neuron lesions than in subjects with an intact innervation of the lower urinary tract (Koyanagi 1978, Nordling et al. 1980).

It may be questioned whether "supersensitivity" of the urethra demonstrated in this way reflects an increased sensitivity to noradrenaline (NA) of the urethral smooth muscle, or whether it can be attributed to other factors.

Patients with lower motor neuron lesions often have a lower intraurethral pressure than normal subjects (Sunder, et al. 1978). It might be that the low starting level of urethral pressure has a more pronounced influence on the amount of pressure increase than an increased smooth muscle sensitivity to the contractant effect of NA. In order to investigate this possibility, knowledge about the dose-response relations for the NA effect on the urethra is desirable. In the present study, the urethral pressure profile was therefore recorded at rest and during continuous i.v. infusion of increasing NA-doses in patients with parasympathetically decentralized lower urinary tract and in normal volunteers. In addition, the effects of the α -adrenoceptor blocker phentolamine on the urethral pressure were compared in the patients and the normal volunteers.

Material and methods

Sixteen patients (Table I), 11 men and 5 women with a mean age of 47 years (range 21–66 years) were investigated with repeated recordings of the urethral pressure profile at rest, during i.v. infusion of NA and after i.v. administration of phentolamine.

All patients had a clinical and cystometrical picture of lower motor neuron lesion. The mean duration was 12 years (range 0.5–50 years). The spinal level of the lesion (Table I) was in all patients below the thoraco-lumbar sympathetic outflow (L₂). Fifteen of the patients had an abnormal EMG-activity at recordings from the urethral and anal sphincteric zones, and flaccidity of the pelvic floor was found in 9 patients. None of the patients had normal sacral reflexes (bulbocavernosus, anal and anocutaneous) and all of them to a variable extent also showed a reduction of the sensitivity in sacral dermatomes. Nine of fifteen investigated patients had a positive bethanechol test, i.e. a detrusor pressure increase of >15 cm H₂O at a bladder filling of approximately 200 ml after bethanechol 0.05mg/kg body weight i.m..

Table I. Clinical data on the sixteen patients with parasympathetic decentralization of the lower urinary tract.

Patient n:o	Age (years)	Sex	Body Weight (kg)	Diagnosis	Vertebral level	Duration (years)
1.	28	♀	57	Vertebral fracture	LI	11
2.	54	♀	60	Prolaps intervert disc	LIV	20
3.	49	♀	65	Vertebral fracture	LII	19
4.	34	♀	58	Subarach. cyst(?)	LI	10
5.	20	♀	61	Myelomeningocele	L–S	20
6.	56	♂	82	Vertebral fracture	LI	28
7.	59	♂	75	Ependymoma medullae spin op	Th–L	20
8.	72	♂	64	Prolaps intervert disc	LIV	1/2
9.	55	♂	90	Prolaps intervert disc	LII–LIV	1
10.	57	♂	67	Chondroma sacralis op	SI–SIV	1/2
11.	54	♂	76	Vertebral fracture	ThXI–XII	1/2
12.	57	♂	63	Spinal stenosis	LIV–V	4
13.	21	♂	69	Vertebral fracture	ThX–XII	1
14.	50	♂	73	Spina bifida	L–S	50
15.	66	♂	60	Vertebral fracture	ThXI–LI	1/2
16.	27	♂	74	Vertebral fracture	LIII	1

Table II. Neurological and urodynamic data on the sixteen patients with para-sympathetic decentralization of the lower urinary tract.

Patient n:o	Emg-findings	Sacral reflexes*			Sensitivity in sacral derma- tomes**	Bladder capacity (ml)	Bethanechol test
		B-C	A	A-C			
1.	Denervation activity	0	0	0	↓	650	Neg
2.	Normal complexes	0	(+)	0	↓ ↓	900	Neg
3.	Neurogenic complexes	0	0	0	↓ ↓ ↓	400	Pos
4.	Neurogenic complexes	0	0	0	↓	650	Pos
5.	Neurogenic complexes	0	0	0	↓ ↓	360	Pos
6.	Neurogenic complexes	(+)	(+)	0	↓ ↓	>1000	Neg
7.	Neurogenic complexes + denervation activity	0	0	0	↓ ↓	850	Pos
8.	Neurogenic complexes	0	0	0	↓ ↓	700	-
9.	Neurogenic complexes	0	0	0	↓ ↓	>1000	Neg
10.	Denervation activity	0	0	0	↓ ↓	680	Pos
11.	Denervation extivity	0	0	0	↓ ↓ ↓	840	Pos
12.	Neurogenic complexes	0	0	0	↓ ↓	500	Pos
13.	Denervation activity	0	0	+	↓ ↓ ↓	750	Pos
14.	Neurogenic complexes	0	0	+(+)	↓	>1000	Pos
15.	Neurogenic complexes	0	0	0	↓	290	Neg
16.	Denervation activity	0	0	0	↓ ↓ ↓	850	Neg

*B-C=bulbocavernosus

A=anal

A-C=ano-cutaneous

**

↓
↓ ↓
↓ ↓ ↓

reduced

strongly reduced

absent

Table III. The maximum urethral pressure (MUP, cm H₂O) in twelve healthy volunteers at rest (MUP₀) and after i.v. administration of noradrenaline to a systolic blood pressure increase of 30 mm Hg (MUP₃₀).

	Subj n:o	Body weight (kg)	MUP ₀	MUP ₃₀	ΔMUP ₃₀
♀	1	62	81	108	27
♀	2	57	73	88	15
♀	3	61	70	81	11
♀	4	54	58	68	10
♀	5	68	92	122	30
♀	6	61	58	62	4
♀	7	57	62	68	6
♂	8	94	88	108	20
♂	9	67	60	78	18
♂	10	70	77	106	29
♂	11	74	65	95	30
♂	12	66	70	95	25
			× 71±3	× 90±5	× 19±3
			(♀ 70±5)	(♀ 85±9)	(♀ 15±4)
			(♂ 72±5)	(♂ 96±5)	(♂ 24±2)

The control group (Table III) comprised twelve healthy volunteers, seven women and five men with a mean age of 35 years (range 26–52 years) without present or previous symptoms from the lower urinary tract and without medication at the time of the investigation.

No patient had clinical signs of lower urinary tract infection on the day of the investigation. Positive urinary cultures ($>10^5$ bacteria/ml) were found in 6 of the patients.

The investigations were approved by the Ethics Committee of the University of Lund.

Investigative procedures

The investigation was performed with the patient in lithotomy position with 100 ml prewarmed (37°C) sterile water instilled into the bladder. All subjects were allowed 30 minutes of rest before the investigation started. Repeated recordings of the urethral pressure profile in each subject were performed before and during continuous i.v. administration of NA, 4 µg/ml. The infusion rate was set with a roller pump I MED 922 (International Medical Group) and stepwise increased every 10–15 minutes according to the following schedule: 15, 30, 45, 60, 90, 120, 150 and 180 ml/h. The blood pressure and pulse were controlled at all levels of infusion, which was stopped at a systolic blood pressure increase of 30 mm Hg above the resting value, usually at an infusion rate of 120–150 ml/h. After the infusion was stopped, phentolamine 5 mg was given i.v. and the urethral pressure profile was again recorded.

In eight of the healthy volunteers NA/s was determined at rest as well as at all levels of NA-infusion and after phentolamine. All blood-samples were immediately centrifuged and the serum was stored at –80°C until analysis of the NA-content using liquid chromatography (Eriksson and Persson 1982) was performed (within two weeks).

The urethral pressure profile was recorded by means of a 7 Ch double microtip transducer catheter (Gaeltec) connected to a Watanabe 796R pen recorder. All recordings were performed with the transducers placed approximately in the three o'clock position. Minor adjustments of the position of the microtip transducers were initially performed to obtain representative and reproducible urethral pressure profiles.

The electromyographic investigations were performed using needle electrodes in the external anal sphincter and in the area of the distal urethral sphincter. The latter electrode was placed, during simultaneous urethroscopy, as near the urethral wall as possible. The amplified signals were displayed on an oscilloscope with storage function. All registrations were photographed. Pathologic activity reflecting damage to the motor innervation of the striated muscle was regarded as present when polyphasic (neurogenic) complexes were found and/or when denervation activity was recorded. The presence of bulbo-cavernosus, anal and anocutaneous reflexes were tested during the EMG-investigation.

Methodological considerations

The method used for measurement of the urethral pressure profile by means of a microtip-transducer is well established (Asmussen and Ulmsten, 1976). In the present investigation, the reproducibility in each individual was good and with only a minor degree of variation in the appearance of the profile. The maximum urethral pressure (MUP) at rest will in the following be called MUP₀, and MUP₃₀ will denote the maximum urethral pressure at a systolic blood

Table IV. The maximum urethral pressure (MUP, cm H₂O) in sixteen patients with parasympathetically decentralized lower urinary tract at rest (MUP₀) and after i.v. administration of noradrenaline to a systolic blood pressure increase of 30 mm Hg (MUP₃₀).

Pat n:o	MUP ₀	MUP ₃₀	ΔMUP ₃₀
♀ 1	22	38	16
♀ 2	43	97	54
♀ 3	30	54	24
♀ 4	51	81	30
♀ 5	58	74	16
♂ 6	11	49	38
♂ 7	51	97	46
♂ 8	24	49	25
♂ 9	18	26	8
♂ 10	57	76	19
♂ 11	58	82	24
♂ 12	67	125	58
♂ 13	45	97	52
♂ 14	41	81	40
♂ 15	35	49	14
♂ 16	57	62	5
	× 39±4	× 68±7	× 29±4
	(♀ 37±5)	(♀ 65±11)	(♀ 28±7)
	(♂ 40±5)	(♂ 70±9)	(♂ 30±5)

pressure increase of 30 mm Hg. ΔMUP₃₀ is the difference between MUP₃₀ and MUP₀.

Results

The maximum urethral pressure at rest (MUP₀) was significantly ($p < 0.001$) lower in both the male and female patients than in the healthy volunteers (Table III and IV). The differences in MUP₀ between men and women within the two groups were small and non-significant.

During NA infusion MUP started to increase already at the lowest rate of infusion (15 ml/hour) in both male and female patients and in male volunteers, and eventually reached a maximum level. However, in the female volunteers, there was a decrease in MUP at the lowest infusion rate and then a dose related increase to the maximum level (Fig 1). There seemed to be no difference in the rate of MUP increase between patients and male volunteers; the rate was slower in the female volunteers. The absolute increase of MUP was greater in the patients than in the volunteers ($p < 0.05$).

Dose-response curves for comparison of the pattern of pressure increase were constructed by setting the maximum increase of MUP as 100%; the results are given in Fig 2a. As can be seen in Fig 2b, there was no displacement to the left of the curve of the patients compared with that of the volunteers. Administration of phentolamine 5 mg i.v. decreased MUP below MUP₀ in both patients and volunteers. As shown in Fig 3 the reduction of MUP (in comparison with MUP₀) was smaller in the male (10±4%) than in the female volunteers (36±2%). This difference was significant ($p < 0.001$). In the patients the decrease was 38±7% in males and 32±6% in females (n.s.).

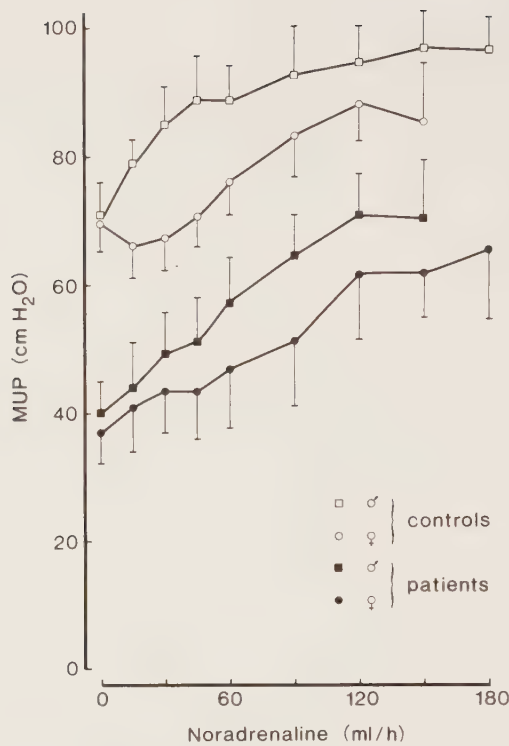


Fig 1. The increase of the maximum urethral pressure (MUP) induced by i.v. infusion of noradrenaline, 4 µg/ml, in patients with parasympathetic decentralization of the lower urinary tract and in healthy volunteers. Bars indicate SEM.

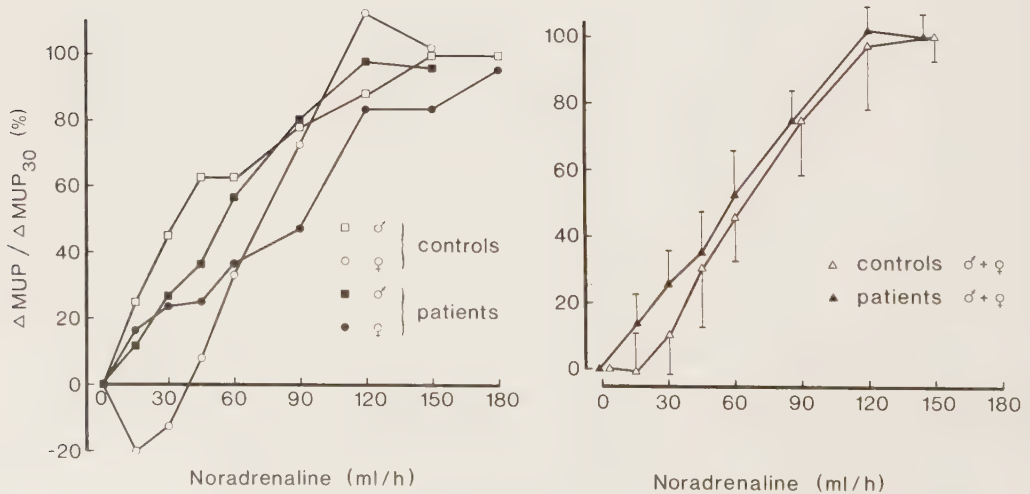


Fig 2a + b. The percentual increase of the maximum urethral pressure (MUP) in the interval MUP_0-MUP_{30} (for explanation, see text) induced by i.v. infusion of noradrenaline, 4 µg/ml, in patients with parasympathetic decentralization of the lower urinary tract and in healthy volunteers. Bars indicate SEM.

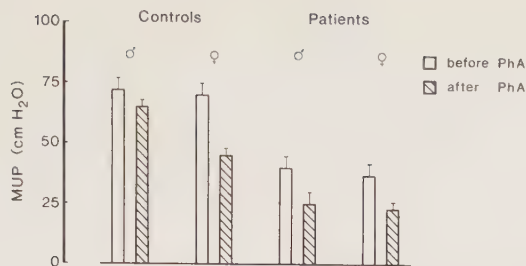


Fig 3. The decrease of the maximum urethral pressure (MUP) induced by phentolamine (PhA), 5 mg i.v., given after previous infusion of noradrenaline in patients with parasympathetic decentralization of the lower urinary tract and in healthy volunteers.

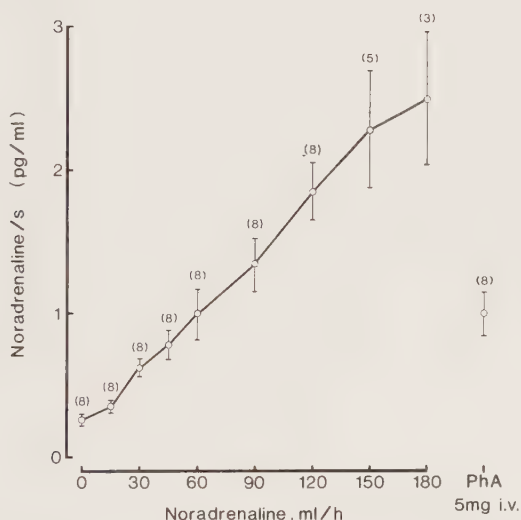


Fig 4. The concentrations of noradrenaline in serum in eight healthy volunteers at different rates of noradrenaline infusion, 4 μ g/ml, and at the time of i.v. administration of 5 mg phentolamine (PhA).

Due to an in average 10 cm H₂O higher bladder pressure in the patient group than in the controls the decrease of the maximum urethral closure pressure (MUCP) was more pronounced in the former group, amounting to approximately 65% of MUCP₀.

The concentrations of NA/s was almost linearly related to the infusion rate of NA (Fig 4). There were no differences in the blood pressure increase between patients and normal volunteers (Fig 5). During the NA infusion there was a decrease in heart rate (from 72 ± 2 to 58 ± 1 beats/min, $p < 0.001$). The only discomfort reported by the patients were palpitations at the highest rates of NA infusion. Flushing and tachycardia were commonly observed after phentolamine injection.

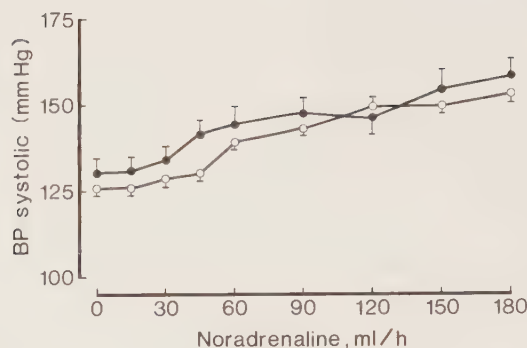


Fig 5. Changes in the systolic blood pressure induced by different rates of noradrenaline infusion (4 μ g/ml) in patients with parasympathetic decentralization of the lower urinary tract (●, $n=16$) and in healthy volunteers (○, $n=12$).

Discussion

We found that in patients with parasympathetically decentralized lower urinary tract, the maximum urethral pressure at rest was significantly lower than in healthy volunteers. This is an agreement with the findings of previous investigators (Koyanagi 1978, Sunder et al 1978). The difference was greater than what could be expected with regard to the different age distribution of the two groups (Enhörning 1961). The absolute increase of the urethral pressure induced by NA was greater in the patients than in the normal individuals. Koyanagi (1978) reported similar results in patients with chronic neurogenic bladders due to lesions at different spinal levels, and so did Nordling et al (1980) in patients subjected to radical hysterectomy. Both Koyanagi (1978) and Nordling et al (1980) used one dose or dose level of α -adrenoceptor agonist (ethylphenylephrine, NA) and suggested on basis of their findings the presence of urethral supersensitivity to α -adrenoceptor stimulation, in analogy with the supersensitivity described for cholinoreceptor stimulating agents in the sacrally decentralized urinary bladder (Lapides et al 1962, Glahn 1970).

Smooth muscle supersensitivity is said to exist when the amount of stimulant required to produce a response is less than normal (Westfall 1981). In order to establish the presence of supersensitivity, it is generally required that a dose-response curve is defined under normal conditions and that this curve is displaced towards lower concentrations of the stimulant in the supersensitive muscle. Supersensitivity after decentralization or denervation usually shows this pattern; an increased maximum response can also be found (Westfall 1981). In the present investigation we found a greater absolute increase of MUP in the patients than in the volunteers during NA infusion, but not even at the highest rate of NA infusion did the MUP of the patients reach the level of MUP at rest in the healthy volunteers. Nor could any consistent leftward displacement of the dose-response curve to NA be demonstrated in the patients. The greater increase of MUP after NA administration in the patients thus seems to reflect a greater residual contraction capacity in the initially (at rest) less activated urethral muscle rather than an increased cellular sensitivity to NA. In support of this view sympathectomy in dogs was not associated with an increased urethral sensitivity to α -adrenoceptor stimulation (Cumes et al 1979).

We have no explanation for the decrease of MUP in comparison to MUP_0 seen in the healthy female volunteers at the lowest rates of NA-infusion. It was probably not related to changes in blood pressure or heart rate since no differences were found in these parameters between the female volunteers and the other groups during the NA-infusion. On the other hand, it cannot be excluded that at least part of the NA-induced increase of the urethral pressure observed in all subjects was attributed to hemodynamic factors.

One patient in the present investigation had a normal EMG from the sphincteric zones. This patient had a clinical picture of a parasympathetically decentralized bladder and also a decrease of the maximum urethral pressure at rest similar to that of the other patients. Such patients have previously been reported by Sunder et al (1978) and Nordling et al (1980). A lesion of the sacral, pelvic innervation of the lower urinary tract with a preserved pudendal motor innervation of the pelvic floor would explain these findings, and such an interpretation is in line with the morphological findings of Gosling et al (1982) showing that the motor innervation of the striated muscle in the distal sphincter mechanism may be mediated via nerve fibers (possibly somatic) in the pelvic nerves.

After administration of phentolamine, there was a significant difference in the reduction of MUP between male and female volunteers; the pressure in the males was reduced by 10% compared to 36% in the females. In the patient group, no such difference could be demonstrated, both males and females responding to phentolamine with a MUP decrease of about 35%. Provided that the response to phentolamine can be taken as a measure of sympathetically mediated tone, it may be speculated that under normal conditions the urethral sympathetic activity is more important for maintaining intraurethral pressure in females than in males.

In the parasympathetically decentralized patients the MUP_0 was low but similar in males and females. It is unclear whether the low pressure can be attributed to the parasympathetic decentralization or to simultaneous loss of somatic innervation of the striated sphincter muscle. The influence of the striated muscle components (striated sphincter and pelvic floor muscles) on the urethral pressure varies in different investigations from almost none to more than 50% of the maximum urethral pressure (see e.g. van Geelen 1983). Neither cholinceptor stimulation nor blockade was shown to have any influence on the intraurethral pressure (Donker et al 1972, Ek et al 1978, Ulmsten & Andersson 1977). These findings do not exclude a transmitter role for acetylcholine in the urethral smooth muscle, but the possibility of a non-cholinergic, non-adrenergic excitatory transmitter should be kept in mind.

Sunder et al (1978) reported that the patients with spinal lesions in the region Th XI–S IV and damage of the parasympathetic efferents had a lower than normal MUP even if the sympathetic outflow was intact, and Koyanagi (1978) found that patients with interruption of the adrenergic nerves to the lower urinary tract and retrograde ejaculation after retroperitoneal lymphadenectomy had a normal MUP. It thus seems as if decrease of MUP is a characteristic of lesions of the pelvic rather than of the hypogastric innervation of the lower urinary tract. If this is correct, an incomplete lesion might result in a less pronounced decrease of the urethral pressure. This might provide an explanation for the variations of the urethral pressure seen in the patient group.

Even if the patients of this investigation had spinal lesions believed to be below the level of the sympathetic outflow, it cannot be excluded that they had some degree of injury also of the sympathetic innervation to the lower urinary tract. Nordling et al (1981), postulating that the parasympathetic innervation had no

importance for the urethral pressure, suggested that the urethral supersensitivity to NA may be a promising test for diagnosing damage of the sympathetic innervation of the lower urinary tract. Their patients had lesions of both the parasympathetic and sympathetic innervation and a lower MUP than normal controls. This may invalidate their conclusions, and it seems doubtful whether the urethral reaction to infused NA can give any reliable diagnostic information on lesions of the autonomic innervation of the lower urinary tract.

Conclusions

No evidence for urethral smooth muscle supersensitivity to NA in patients with parasympathetically decentralized lower urinary tract was found in the present investigation. It cannot be excluded that the decreased MUP observed in patients with lesions of the sacral motor innervation to the bladder and urethra reflects a loss of a contribution of the parasympathetic innervation to the regulation of normal pressure in both male and female urethra. The adrenergic nervous influence may be of greater importance for the maintenance of resting urethral pressure in women than in men. The urethral pressure response to α -adrenoceptor stimulation does not seem to be a reliable test for diagnosing damage of the autonomic innervation of the lower urinary tract.

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EFFECTS OF PRAZOSIN ON ISOLATED HUMAN URETHRA AND IN PATIENTS WITH LOWER MOTOR NEURON LESIONS¹

K.-E. ANDERSSON,² A. EK, H. HEDLUND, AND A. MATTIASSON

Departments of Clinical Pharmacology and Urology, University Hospital of Lund, Lund, Sweden

ABSTRACT

In isolated human urethra, prazosin competitively inhibited contractions induced by noradrenaline, but had no effect on contractions elicited by potassium (127 mM), suggesting an action solely on α -adrenoceptors. Seven patients with lower motor neuron lesions and micturition disturbances were treated with 2 mg of prazosin twice daily. Five were investigated by simultaneous urethrocystometry before and during treatment. Prazosin reduced the intraurethral pressure, the intravesical pressure during bladder filling, and autonomous bladder waves. Voiding and incontinence improved in five patients. In one, voiding was facilitated, but continence deteriorated, and in one, no clinical effects were observed. In all patients, residual urine decreased. There were no side effects, except one case of nasal congestion. It is concluded that prazosin effectively reduces α -adrenoceptor mediated hyperactivity in the smooth muscle of the lower urinary tract, and that it may be an effective treatment of micturition disturbances in patients with lower motor neuron lesions.

It is well established that the smooth muscle of the bladder neck and the urethra in man is sympathetically innervated (1), and that there is a significant α -adrenoceptor mediated contribution to the intraurethral pressure (2, 3). In patients with lower motor neuron lesions and micturition disturbances caused by increased intraurethral resistance to flow during voiding, favorable therapeutic results have been obtained by use of α -adrenoceptor blocking agents (4-9) which have also been shown to improve voiding in patients with benign prostatic hyperplasia (10-13). However, side effects are not infrequent during treatment with phenoxybenzamine (10, 14), the α -adrenoceptor blocker most commonly used in these disorders.

Prazosin is an antihypertensive drug differing from previous α -adrenoceptor blockers by having a selective action on post-synaptic α -adrenoceptors (15, 16). When used in hypertensive patients, few side effects of the drug have been reported (16, 17). However, a few cases of stress urinary incontinence have been observed during prazosin treatment of hypertension (18, 19), suggesting that the drug might be therapeutically effective in patients with an increased urethral resistance to flow during micturition.

We investigated the effects of prazosin on isolated human urethra and on patients with lower motor neuron lesions and micturition disturbances.

MATERIALS AND METHODS

In vitro

Tubal segments from the urethra were obtained from five men, aged 46 to 75 years, who were undergoing cystourethrectomy because of malignancy of the bladder. Preoperative radiological treatment (4×500 rad) had been given to four of these patients.

Immediately after removal of the bladder and urethra an

bloc, the urethral tissue specimens (taken mainly from the juxtacollicular parts of the organ) were placed in chilled Krebs solution and transported to the laboratory. The tubal specimens were cut open, the mucosa removed, and the circular smooth muscle layer carefully dissected into strip preparations approximately 1.0 to 2.0 mm wide, 0.5 to 1.0 mm thick, and 8.0 to 10.0 mm long. Within 1 hour after removal of the tissue specimens, the preparations were mounted in 30 ml, thermostatically controlled organ baths filled with Krebs solution kept at 37°C and bubbled with 5 per cent CO_2 and 95 per cent O_2 . Isometric tension was recorded by means of Grass Ft O3 transducers connected to a Grass model P 7 polygraph. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.6, CaCl_2 2.5, MgSO_4 1.15, NaHCO_3 24.9, KH_2PO_4 1.5, glucose 5.5; pH was 7.40. In some experiments, a depolarizing solution containing 127 mM potassium was obtained by exchanging sodium in the normal Krebs solution for equimolar amounts of potassium. All solutions were prepared on the day of the experiment. The chemicals used were of analytical grade. The initial tension of the preparations was set at approximately 5 mN. The preparations were allowed to relax, and a stable tension level was obtained before the experiment started. A total of 39 preparations were used. Reproducible responses to potassium and noradrenaline were not obtained in three; these preparations were discarded. Drugs used were (-) noradrenaline bitartrate (Sigma Chemical Company), phentolamine methane sulphone (Ciba Geigy), prazosin hydrochloride (Pfizer). The drugs were injected into the bath. Concentrations given are final bath concentration.

In vivo

Five women and two men with lower motor neuron lesions and autonomous bladder, aged 14 to 60 years, were studied (Table 1). All had anesthesia within sacral dermatomes and areflexia of the anal sphincter. The women were investigated by simultaneous urethrocystometry and recording of the urethral closure pressure profile (UCPP) with the technique described by Asmussen and Ulmsten (20, 21). The intraurethral and intravesical pressures and the difference between these, i.e., the urethral closure pressure, were recorded simultaneously by means of a dacron catheter (o.d. 4.6 to 6.0 mm) containing

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² Address requests for reprints to: Professor Karl-Erik Andersson, Department of Clinical Pharmacology, EA-blocket, University Hospital of Lund, S-221 85 Lund, Sweden.

two microtransducers, one at the tip, and the other 6 cm proximally. By the tip transducer, the intravesical pressure was recorded continuously, and by the proximal transducers the UCPP was recorded at bladder volumes of 100 and 300 ml in the lithotomy position and at 300 ml in the erect position. The bladder was filled with body warm saline at a rate of 25 ml per min through a thin (o.d. 1.3 mm) urethral catheter. When recording the UCPP, the recording catheter was removed at a constant rate of 5 mm per sec through the urethra. The urodynamic investigation was performed on three occasions: (i) before treatment, (ii) 1 hour after oral administration of norephedrine (oral solution) 1 mg per kg of body weight and immediately after intravenous injection of 5 mg of phentol-

TABLE 1. Patients with lower motor neuron lesions treated with prazosin

Patient No.	Sex	Age	Diagnosis	Clinical Symptoms
		yr		
1	female	19	Myelomeningocele	poor voiding, severe incontinence
2	female	14	Myelomeningocele	difficult voiding, mild incontinence
3	female	57	Arachnoiditis, flaccid paraplegia	poor voiding, severe incontinence
4	female	44	Arachnoiditis	difficult voiding, mild incontinence
5	female	46	St. p. op. protruding lumbar disc.	unable to void, self-catheterization
6	male	60	Fract. vert. L I, flaccid paraplegia	unable to void, indwelling catheter
7	male	18	Fract. vert. L I, flaccid paraplegia	unable to void, indwelling catheter

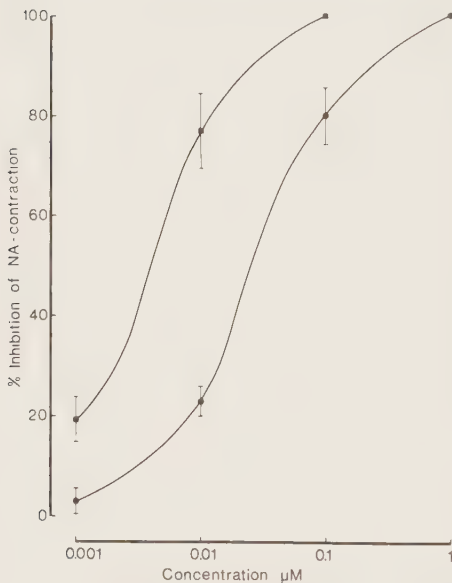


FIG. 1. Concentration dependent, inhibitory effects of prazosin (squares) and phentolamine (circles) on contractions induced by noradrenaline $2 \mu\text{M}$ in isolated human urethra. Mean $\pm$ SE, $n = 5-6$.

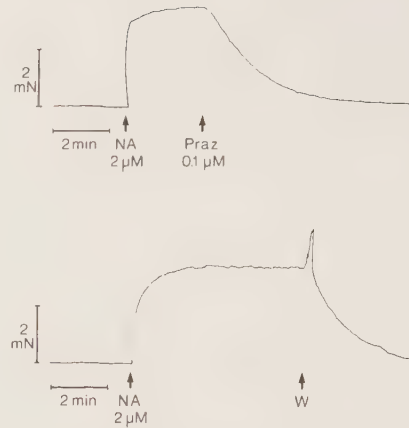


FIG. 2. Relaxant effect of prazosin (Praz) on a noradrenaline (NA) induced contraction in isolated human urethra. W = wash.

amine (Regitin, Ciba Geigy), and (iii) after more than 2 weeks of treatment with prazosin (Peripress, Pfizer) 2 mg twice daily. To avoid side effects ("first dose phenomenon," 16, 17) the dose of prazosin was successively increased during the first week from 0.5 mg twice daily to maintenance dose. The two male patients had indwelling urethral catheters, and were not urodynamically investigated.

RESULTS

In vitro

The preparations were contracted by noradrenaline in a concentration related way. These contractions were competitively antagonized by prazosin. Figure 1 shows the concentration-response curves obtained by the addition of prazosin and phentolamine in various concentrations to preparations 10 min before contractions were induced by $2 \mu\text{M}$ noradrenaline. This noradrenaline concentration was found to give a submaximum but reproducible contraction. Addition of the α -receptor blockers inhibited the noradrenaline induced contraction in a concentration dependent way. Compared with phentolamine, prazosin was the more potent drug on a molar basis, being approximately ten times more effective than phentolamine. Preparations contracted by noradrenaline (Fig. 2) could be completely relaxed by prazosin. Prazosin in concentrations up to $0.1 \mu\text{M}$ (a concentration completely inhibiting contractions induced by $2 \mu\text{M}$ of noradrenaline) had no appreciable effect on contractions elicited by 127 mM of potassium, neither when added to potassium contracted preparations, nor when added 10 min before the contraction was evoked.

In vivo

Effects on intraurethral pressures. In all examined women norephedrine increased and prazosin decreased the maximum urethral pressure (MUP) and the maximum urethral closure pressure (MUCP). These effects were seen at all the tested bladder volumes, and in both lithotomy and erect positions (Table 2). When phentolamine was given i.v. immediately after the norephedrine induced changes in urethral pressure had been recorded at a bladder volume of 300 ml, there was a fall of both MUP and MUCP to levels well below those of the controls (Table 2). The pressure changes comprised the whole extent of

the UCPP and were not confined to any particular segment of the urethra (Fig. 3). The changes in urethral pressure were statistically significant ($P < 0.02$) except in the erect position. In two women with myelomeningocele and trabeculated hypertonic bladder, prazosin decreased intravesical pressure during filling, and reduced the "autonomous waves" (22) i.e., the longstanding, weak detrusor contractions that could be recorded. In all patients, residual urine decreased.

Effects on clinical symptoms. Five of the seven patients treated with prazosin improved during treatment (Table 3). One woman who managed by intermittent self catheterization was able to void with negligible residual urine. She remained continent. Voiding and continence improved in two women with considerable residual urine and severe incontinence, whereas two with little residual urine and mild incontinence were not improved by prazosin. Two men with flaccid paraplegia, who had had indwelling urethral catheters for 3 and 6 months, respectively, could manage without catheters and remained continent with negligible residual urine during prazosin treatment.

DISCUSSION

The present results showed that prazosin effectively counteracted noradrenaline induced contractions in isolated human urethra in concentrations that had no appreciable effects on contractions evoked by potassium. These findings suggest that the prazosin effect was exerted solely by blockade of α -adrenoceptors and not by an unspecific smooth muscle relaxant action which in some studies has been attributed to the drug (23). Prazosin also reduced the intraurethral pressure in patients with lower motor neuron lesions, decreased the intravesical pressure during bladder filling, and inhibited autonomous bladder waves. Sundin et al. (9) found evidence for the existence of α -adrenoceptors in the bladder of patients with lower motor neuron lesions. Normally, α -adrenoceptors cannot be shown in the bladder, but in patients with autonomous bladder they may be responsible for the autonomous waves seen in the cystome-

trogram during bladder filling (9, 22). An increased α -adrenoceptor function may be found also in the urethra. Thus, Koyanagi (24) observed an increased response to α -adrenoceptor stimulation as judged by effects on the urethral pressure profile in patients with chronic neurogenic bladder, suggesting a denervation supersensitivity of the urethra. It is well known that patients with lower motor neuron lesions and autonomous bladder do not have a normal micturition pattern. Not only do

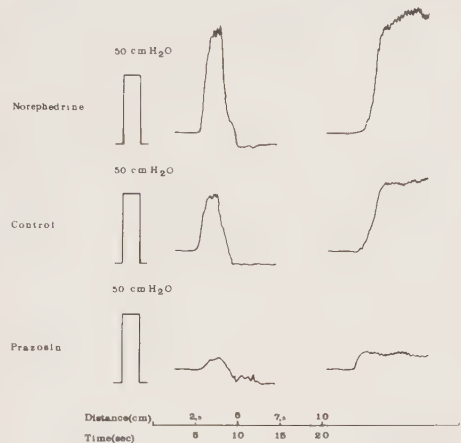


FIG. 3. Effects of norephedrine and prazosin on the urethral pressure profile in one of the patients. The right part of the figure shows recordings obtained with the urethral catheter maintained at the region of maximal urethral pressure.

TABLE 2. Maximum urethral pressure (MUP) and maximum urethral closure pressure (MUCP) at different bladder volumes in five women with lower motor neuron lesions.^a

Bladder volume	100 ml						300 ml						300 ml (erect)					
	1	2	3	4	5	mean	1	2	3	4	5	mean	1	2	3	4	5	mean
Patient																		
MUP																		
Control	57	69	39	54	63	56.5	54	64	38	56	56	53.6	83	84	69	62	83	76.2
Norephedrine	95	97	45	78	82	79.4	91	87	45	84	90	79.4	96	120	72	84	110	96.4
Phentolamine							34	48	33	24	37	35.2						
Prazosin	23	39	33	41	26	32.4	26	37	31	39	24	31.4	44	38	60	58	61	52.2
MUCP																		
Control	48	60	26	48	42	44.8	44	30	24	48	35	36.2	49	35	38	34	48	40.8
Norephedrine	86	82	31	68	67	66.8	52	49	31	73	68	54.6	68	50	35	57	72	56.4
Phentolamine							14	20	18	16	16	16.8						
Prazosin	15	24	18	30	20	21.4	8	4	12	31	22	15.4	14	10	15	30	27	19.2

^a Pressures expressed in cm H₂O

TABLE 3. Effects of prazosin treatment

Patient No.	Residual urine (ml)		Blood pressure:		Clinical Effects	Side Effects
	Before	During	Before	During		
	ml	ml	mm Hg	mm Hg		
1	200	15	120/70	115/75	Improved voiding, almost continent	None
2	40	30	110/70	110/75	Voiding facilitated, deterioration of continence	Nasal congestion
3	200	40	150/80	140/90	Improvement of voiding and continence	None
4	60	30	130/80	130/75	None	None
5	>400	60	130/80	140/80	Able to void, continent	None
6	>400	40	160/80	160/90	No need of catheter, continent	None
7	>400	15	115/70	110/70	No need of catheter, continent	None

they lack detrusor contractions of the micturition type, but they are also unable to relax their urethra in a normal way at voiding. This may give rise to considerable residual urine with serious consequences for the upper urinary tract and renal function, because the patients also often have trabeculated, hypertonic bladders. In addition, urinary incontinence is common in these patients. The incontinence may be of two types often occurring simultaneously: (i) stress incontinence caused by paralysis of the muscles of the pelvic floor, or (ii) involuntary voiding due to variation in detrusor tone, at cystometry recorded as autonomous waves. Both these types of incontinence may be aggravated or start when the patient has a considerable residual urine.

Thus, there are several reasons why α -adrenoceptor blockade by reducing residual urine should improve continence as well as save renal function in patients with lower motor neuron lesions. The effectiveness of these drugs has also been reported by several investigators (4-9, 25). In the present series five out of seven patients benefited from treatment with prazosin, a success rate comparable to that obtained with phenoxybenzamine, the α -adrenoceptor blocker most commonly used. This drug has however several disadvantages. It causes an irreversible blockade of α -adrenoceptors which may produce reflex tachycardia and a postural hypotension of long duration. Other side actions such as dizziness, fainting and lethargy are not uncommon (10, 12, 14, 25). Prazosin is a competitive antagonist which, possibly due to the lack of effect on presynaptic α -adrenoceptors, produces little effect on heart rate. Side effects of prazosin are generally considered unimportant except for the so called "first dose phenomenon" (16). This acute hypotensive effect, observed in hypertensive patients, can be avoided if the initial dose of the drug is kept low. In the present patients the drug was well tolerated but further experience from patients with lower motor neuron lesions is needed. The effectiveness of prazosin in lowering the intraurethral pressure, which also has been confirmed in studies on animals (26), suggests that it may be a favorable alternative in the treatment of patients with lower motor neuron lesions and voiding disturbances.

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Supersensitivity to Carbachol in the Parasympathetically Decentralized Feline Urinary Bladder

By

Anders Mattiasson, Karl-Erik Andersson, Christer Sjögren, Torsten Sundin & Bengt Uvelius

From the Departments of Urology, Clinical Pharmacology and Physiology, University Hospital, Lund, Sweden

Abstract

We investigated the concentration-response relations for carbachol, the morphological characteristics and the mechanical properties of feline detrusor strips from ¹normal cats, cats subjected to ²parasympathetical, sacral decentralization, ³urinary diversion followed by parasympathetical, sacral decentralization and ⁴urinary diversion only. Hypertrophy of the detrusor and supersensitivity to carbachol (a decrease of EC_{50}) were found only after parasympathetical decentralization. No hypertrophy developed and no change in the EC_{50} -value for carbachol was found if urinary diversion preceded the parasympathetical decentralization. A decreased ability of force production per unit cross sectional smooth muscle area was found in the decentralized bladders compared to the controls. However, the total ability of force production and hence also pressure production of the decentralized bladders would be expected to be enhanced due to a 4–5 fold increase of bladder weight (mainly muscle mass). No differences in the active length-tension relations were found in the four groups. It is suggested that the parasympathetical decentralization per se does not give rise to detrusor hypertrophy or increased sensitivity to carbachol. Provided that the situation in man is comparable to that in the cat, it might be that the supersensitivity test a.m. Lapidus-Glahn reflects the presence of detrusor hypertrophy rather than the presence of a neurogenic lesion.

Introduction

In the parasympathetically decentralized, neurogenic urinary bladder two characteristic findings are supersensitivity to cholinceptor-stimulating agents and hypertrophy of the bladder wall (1, 2, 3, 4). It is not known whether the supersensitivity is due to the decentralization per se or if it is secondary to changes in the lower urinary tract induced by the decentralization, e.g., bladder wall hypertrophy associated with impaired ability to empty the bladder (2, 3).

Supersensitivity of the detrusor in a clinical sense is said to be present if a certain minimum increase of the detrusor pressure is recorded after administra-

tion of a cholinceptor-stimulating agent (4, 5). It might, however, be that this pressure increase is secondary to changed mechanical properties of the decentralized, hypertrophic detrusor, rather than to an increased sensitivity to cholinceptor-stimulating agents at the receptor level. The present investigation was performed to study whether true supersensitivity to cholinceptor-stimulating agents develops in the parasympathetically decentralized urinary bladder, and/or whether possible changes of the mechanical properties can explain the increased pressure production after administration of a cholinceptor stimulating agent.

Material and methods

Morphological characteristics, mechanical properties (i.e. passive and active length-tension relations and force production per unit cross sectional smooth muscle area), and the concentration-response relations for carbachol were investigated in feline detrusor strips. Preparations were obtained from four groups of animals 1) normal cats ($n=4$), cats subjected to 2) parasympathetical decentralization ($n=6$), 3) urinary diversion followed by parasympathetical decentralization ($n=4$), and 4) urinary diversion only ($n=2$). Sixteen cats weighing 3 to 5 kg, 8 males and 8 females, were used in the study.

Surgical procedures

The cats were anaesthetized with pentobarbital (Nembutal, 30 mg/kg i.p.) and had spontaneous respiration. All operations were performed with microsurgical technique. The parasympathetic decentralization was performed essentially as described by Carlsson and Sundin (6). Via a midline incision in the back laminectomy was performed, the dura opened, and the ventral sacral roots S_1-S_{III} were divided bilaterally. Silver clips were applied on the central root-endings to prevent regeneration. Urinary diversion was performed as a uretersigmoideocutaneostomy with an approximately 3 cm long conduit. The anastomosis between the ureters and the conduit was constructed according to the "fish-mouth principle" with dextron 8-0. In the cats operated with both urinary diversion and sacral root division the former operation was performed approximately three weeks before the decentralization. In cats subjected to decentralization the bladders were emptied regularly by external compression. All cats were closely observed and at any sign of infection, urinary cultures were performed. Upper urinary tract infection with positive urinary culture was seen in the postoperative period in 5 of 6 cats operated with urinary diversion. The infection was treated with streptomycin i.m. for three days and subcutaneous injection of isotonic glucose solution. At the time of sacrifice of the animals urinary cultures taken from the urinary bladder were negative in all cats, and no macroscopical signs of infection were noted. In three of the animals operated with urinary diversion, positive urinary cultures were found. At the time of sacrifice creatinine in serum was $96 \pm 17 \mu\text{mol/l}$ in the group of animals subjected to urinary diversion ($n=6$) and $92 \pm 6 \mu\text{mol/l}$ in the normal cats ($n=4$) while the value for the cats subjected only to sacral decentralization was $87 \pm 9 \mu\text{mol/l}$ ($n=6$). Ten to twelve weeks after the last operation, the animals were anaesthetized and the bladder was removed together with the proximal urethra. The emptied bladder was dissected free from surrounding fat and connective tissue and weighed.

Preparation

The bladder was filled with 10 ml Krebs solution via a needle through the bladder wall. One piece of bladder tissue with a measured in situ length of 10 mm and a width of about 6 mm was taken from the ventral part of the bladder dome for mechanical investigation. The preparations were taken in the longitudinal fiber direction of the detrusor muscle and dissected into two strip preparations. When the mechanical investigation was completed the strips were embedded for light microscopy (see below). For determination of the carbachol concentration response relations, tissue was taken from each half of the bladder dome, and two to four strip preparations, approximately $1 \times 2 \times 8$ mm, were dissected from each bladder half.

Carbachol concentration-response relations

The detrusor strips were suspended in 10 ml water mantled, thermostatically controlled organ baths containing Krebs solution (37°C) bubbled with a mixture of 5% CO_2 and 95% O_2 giving a pH of approximately 7.4. The preparations were allowed to equilibrate for about one hour and the final tension was adjusted to 5–8 mN. The isometric tension of the preparations was recorded by means of Statham FT 03C force transducers connected to a Grass 7D polygraph. The contractile capacity of each preparation was controlled by exposure to high K^+ solution (for composition, see below). Carbachol was added cumulatively, starting with a concentration of $5 \times 10^{-8}\text{M}$.

Length-tension relations

The preparations were mounted in a 50 ml water mantled, thermostatically controlled (37°C) organ bath containing Krebs solution. The preparations were stretched to the in situ length (10 mm, L_{10}). They were allowed to equilibrate for 30 minutes. The Krebs solution was then changed to a nominally Ca^{2+} -free Krebs solution and the strips were exposed for 30 minutes.

The preparations were contracted for 5 minutes in high K^+ -solution containing Ca^{2+} , and after another 10 minutes in Ca^{2+} -free solution (which was enough to induce complete relaxation), they were again contracted in high K^+ -solution. After relaxation the strips were passively shortened to 5 mm ($0.5 L_{10}$), and then stimulated again. The procedure was repeated and passive and active tensions were determined for $0.5 L_{10}$, $0.7 L_{10}$, $0.85 L_{10}$, L_{10} , $1.15 L_{10}$, $1.30 L_{10}$ and $1.45 L_{10}$. After this had been accomplished, the length of the preparations was readjusted to L_{10} , and after 15 minutes the Ca^{2+} -free Krebs solution was changed to a fixative (5% glutaraldehyde in 100 mM cacodylate buffer, pH 7.4).

Morphological investigation

After 2 hours in glutaraldehyde, the preparations were postfixated in 2% OsO_4 , dehydrated and embedded in Vestopal-W (Jaeger, Switzerland). After polymerization transverse sections ($1\text{--}2\ \mu\text{m}$ thick) were cut with glass knife. The sections were stained with 1% methylene blue and 1% azur II in a 1% aqueous solution of borax, examined and photographed in a light microscope. Muscle cross-sectional area was determined by weighing cut-outs of the micrographs.

Solutions and drugs

The Krebs solution had the following composition (mM): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11. The high K⁺-solution contained 124 mM K⁺, Na⁺ being reduced correspondingly. Nominally Ca²⁺-free solution was obtained by omitting CaCl₂ in the Krebs solution. All solutions were prepared the day of the experiment. Double distilled water and analytical grade chemicals were used.

Carbachol (carbamyl-choline chloride) was obtained from EDH Chemicals Ltd.

Statistical analysis

Statistical comparisons were performed using computer analysis of variance. All results are given as mean $\pm$ standard error of mean (SEM), unless otherwise stated. The number of observations (n) is given in parenthesis. The mean $\pm$ SEM of all observations were used for construction of concentration-response curves given in the figures. For statistical comparisons the man values obtained for preparations (n=2–4) from each bladder half were used.

Results

Morphologically verified hypertrophy of the detrusor was found only in the parasympathetically decentralized cats. The bladder weight in this group was 13.4 ± 2 g (n=6) while the weight for the control bladders was 2.9 ± 0.4 g (n=4). The weights of the bladders in cats subjected to urinary diversion only and urinary diversion followed by parasympathetical decentralization were 2.5 and 4.4 g (n=2), and 3.3 ± 0.3 g (n=4), respectively. No macro- or microscopical signs of inflammatory reaction or degenerative changes were found in any bladder investigated.

Carbachol concentration-response relations

The concentration-response curves for carbachol are shown in figs. 1–3 and the EC₅₀ values and slope factors are given in table I. A significantly ($p < 0.02$) increased sensitivity to carbachol was found in the parasympathetically decentralized group compared to the normal bladders. The shape of the concentration-response curve was not changed and the EC₅₀ value decreased from 0.41 to 0.20 μ M (fig. 1, Table I). In preparations from cats subjected to urinary diversion and parasympathetical decentralization the slope of the concentration-response curve tended to be steeper (non-significant) than in the controls; the EC₅₀ value was not significantly different (fig. 2). Comparison between the group subjected to parasympathetical decentralization and the group subjected to urinary diversion followed by parasympathetical decentralization revealed no difference in the slope of the concentration response

Table I. EC₅₀ (mean $\pm$ SEM) and slope factors for the carbachol concentration-response curves.

	EC ₅₀ (μ M)	Slope Factor
Controls	0.41 ± 0.01	1.0
Decentr.	0.20 ± 0.01	1.12
Urin. div. + decentr.	0.35 ± 0.01	1.23
Urin. div.	0.42 ± 0.01	1.06

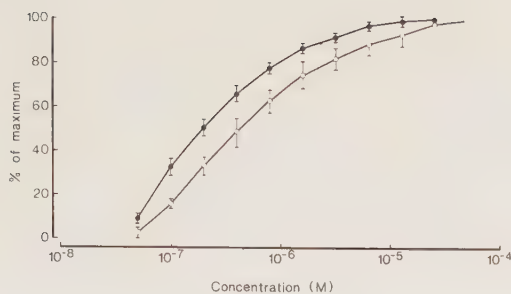


Fig 1. Carbachol concentration-response curves for detrusor strips ($n=24-31$) from normal cats (○) and from cats subjected to parasympathetic decentralization (●).

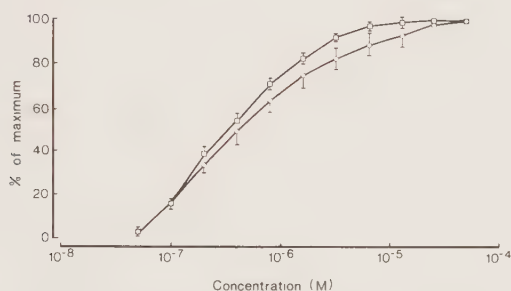


Fig 2. Carbachol concentration-response curves for detrusor-strips ($n=24-27$) from normal cats (○) and from cats subjected to urinary diversion followed by parasympathetic decentralization (□).

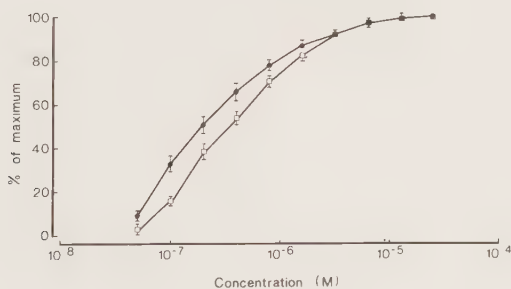


Fig 3. Carbachol concentration-response curves for detrusor-strips ($n=24-27$) from cats subjected to parasympathetic decentralization (●) and from cats subjected to urinary diversion followed by parasympathetic decentralization (□).

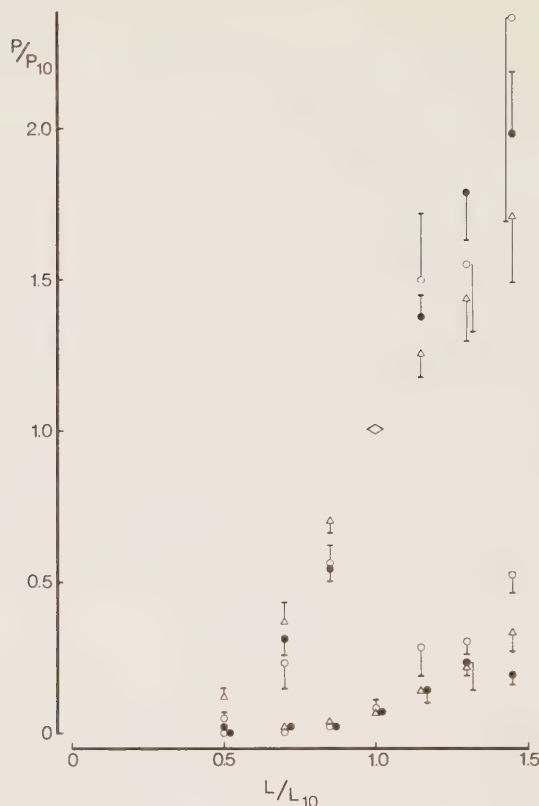


Fig 4. Active (upper) and passive (lower) length (L)-tension (P) relations for detrusor-strips from normal cats ($\triangle$) and cats subjected to parasympathetical decentralization ($\bullet$), and urinary diversion followed by parasympathetical decentralization ($\circ$). The force produced at $L_{10}=1.0$ (for explanation, see text). The L_{10}/P_{10} reference point is marked $\diamond$.

curves, but the EC_{50} value was significantly ($p<0.05$) lower in the former group (fig. 3). Concentration-response curves from the two cats subjected to urinary diversion only did not differ from those of the controls.

Length-tension relations

Passive and active (K^+ contractures) force values were obtained at preparation lengths between $0.5 L_{10}$ and $1.45 L_{10}$ as described in Methods. The results are given in fig. 4. The force (expressed relative to active force at L_{10}) was similar at each length for bladder strips from animals in the control group and from animals subjected to parasympathetical decentralization or to urinary diversion followed by parasympathetical decentralization. Length-tension relations were also determined for four bladder strips from the two cats operated with urinary diversion only. Whereas the passive force had increased in these strips, length-active force relations were similar to those in the other groups illustrated in fig. 4. In all groups, the cross-sectional area of the detrusor strips was measured on photo-micrographs and was found to vary between 0.095 and 0.614 mm^2 . Force per unit cross-sectional muscle area at L_{10} could be calculated for each individual strip. The results are given in Table II. Compared with the control group force per area was significantly ($p<0.001$) lower in the other groups.

Table II. Force/unit cross sectional muscle area

	mN/mm ²	n
Controls	214±43	8
Decentr.	100±16	12
Urin. div. + decentr.	76±27	7
Urin. div.	60±15	4

Morphological investigation

The microscopical investigation revealed an increased density of smooth muscle bundles in the detrusor strips from bladders subjected to parasympathetical decentralization only. The size of the cross-sectioned cells in this group was considerably greater than in the other groups investigated, indicating hypertrophy of the smooth muscle cells.

Discussion

The present studies revealed detrusor hypertrophy and an increased sensitivity to carbachol in cats subjected to parasympathetic decentralization only. If urinary diversion preceded this intervention no hypertrophy developed. The EC₅₀ value for carbachol was not different from that of the controls, but the slope of the curve tended to increase. This may favour the view that changes secondary to the neurogenic lesion and not the neurogenic lesion per se induced the increased sensitivity to carbachol in the parasympathetically decentralized urinary bladder, and suggests that bladder hypertrophy is a major but perhaps not the only factor associated with the increased response to carbachol.

The lack of detrusor hypertrophy in cats subjected to urinary diversion before parasympathetical decentralization is in agreement with the findings of Carpenter (2). The detrusor hypertrophy found in cats subjected to parasympathetical decentralization only, reflected both by the increase in bladder weight and in size of the smooth muscle cells, is probably related to the impairment of bladder emptying (3). However, Pompino et al (7) could not exclude that the thickening of the bladder wall found in the parasympathetically decentralized rabbit urinary bladder was secondary to inflammatory changes caused by urinary infection. Also Carpenter (2) described oedematous changes of the bladder wall in cats subjected to parasympathetical decentralization of the lower urinary tract. In the present investigation no macro- or microscopical signs of inflammatory reaction of the bladder wall were found, probably excluding such an explanation for the bladder wall thickening.

No differences in the length-active tension relations were found in the different groups investigated. The ability of force production per unit muscle cross sectional area was approximately halved in the decentralized bladders compared to the controls. This has previously been shown in other types of hypertrophic smooth muscle (8). In the bladders subjected to urinary diversion the ability of force production was even less. As the bladder weight in the decentralized group, however, had increased almost 5-fold compared to the controls (mainly an increased muscle mass) the total ability of force- and hence also pressure production would be expected to be considerably greater in the hypertrophic than in the control bladders. Whether the parasympathetical decentralization was causal for the development of detrusor hypertrophy in any

other way(s) than by an impaired ability of bladder emptying was not revealed by the present investigation.

Provided that the situation in man is comparable to that in the cat, it might be that it is not only the increased sensitivity to cholinceptor stimulating agents in the parasympathetically decentralized, hypertrophic bladder, but also the increased ability of total force production that explain the supersensitivity (in a clinical sense) seen in patients with sacrally decentralized lower urinary tract. As the increased sensitivity to carbachol at the cellular level seemed to be related to the impaired bladder function and to the detrusor hypertrophy, and not to the neurogenic lesion per se, it is not surprising that the clinical supersensitivity tests are often found to be nonspecific (9). It might even be that the tests reflect the presence of detrusor hypertrophy rather than the presence of a neurogenic lesion.

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